CONCEPTUS-RELATED CHANGES
OF THE ENDOCRINE AND PHYSIOLOGICAL DYNAMICS
DURING THE PERIPARTURIENT PERIOD IN CATTLE

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1984

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Dedicated to my mother

ACKNOWLEDGEMENTS

One does not arrive at the stage of presenting a Ph.D. dissertation at the University of Florida all alone, and I would like to pay tribute to the assistance I have received in order to reach the goal I set some four years ago.

I would like to express my sincerest gratitude to Dr. William W. Thatcher, chairman of my supervisory committee and my mentor. His guidance, criticism and never-ending enthusiasm were a constant source of inspiration and made this research a memorable experience. I was very fortunate to learn from such a leader in the scientific community, and I thank him for the friendship and support he has extended to me throughout my research program.

I would like to thank Dr. Fuller W. Bazer for his friendship and guidance particularly during the last phase of this project. He has played a major role in the success of my program and education in general.

Special thanks are expressed to Dr. Marteen Drost for his time and assistance throughout the course of this research. His invaluable surgical skills, which were surpassed only by his extreme patience, made possible the realization of some critical phases of this research.

I am grateful to Dr. Donald Caton for advice and surgical training and for bringing his unique perspective to this research.

The advice, encouragement and friendship of Dr. Robert J. Collier, even from a distance, are acknowledged and greatly appreciated.

I am indebted to Dr. Donald H. Barron for stimulating discussions and for accepting to serve as member of my committee during Dr. Collier's sabbatical leave.

Sincere appreciation is also extended to those noncommittee faculty who have aided in various aspects of this experience. Such acknowledgement is expressed especially to Dr. C.J. Wilcox for providing me with a strong statistical background. The friendship and encouragement of Drs. Herbert H. Head and David K. Beede and the support of Drs. H.H. (Jack) Van Horn and Roger P. Natzke are acknowledged. I also wish to express my gratitude to post-doctoral fellows, Drs. Greg S. Lewis and David Wolfensen for their friendship, support and assistance and to Dr. James W. Lauderdale of the UpJohn Company for positive criticism and support during this research. All members of the interdisciplinary reproductive biology group at the University of Florida are gratefully acknowledged.

My thanks must also be addressed to Agriculture-Canada for their financial support. Without such support, this study would not have been realized.

Very special thanks are extended to fellow graduate students, Dallas Foster and Jeff Knickerbocker. Their friendship and invaluable help provided during some laborious surgeries will always be remembered.

Thanks are also expressed to Dr. Frank F. Bartol, Joachim Moya, Joan Curl, Paul Mallonee, Paul Schneider, Lokenga Badinga, Chuck Wallace and Marlin Dehoff. The help and skill of Drs. George Haibel and Steven Hopkins, residents at the College of Veterinary Medicine, are gratefully acknowledged.

I wish to express my gratitude to Carol Underwood for her help in sample collection and laboratory analyses. My thanks are addressed also to Larry (Israel) Grayhills and Marie-Joelle Thatcher for their technical help. The assistance in handling of animals of Mr. Tom Bruce, who more than often went beyond the call of duty, and of Mr. Austin Green deserves acknowledgements.

I would like to acknowledge Cindy Zimmerman for typing this manuscript.

Finally, and I have saved the best until the end, to my wife and best friend Louise, who has bravely endured the hardships of leaving family and friends, has adapted to a new language and culture and has supported and encouraged me in every way in order to enable me to complete this degree, I express my sincerest love and gratitude. The opportunity that I have to share with her this period of my life makes it more than a simple academic learning experience.

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Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

CONCEPTUS-RELATED CHANGES OF THE ENDOCRINE AND PHYSIOLOGICAL DYNAMICS DURING THE PERIPARTURIENT PERIOD IN CATTLE

By

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August, 1984

Chairman: William W. Thatcher
Major Department: Animal Science

Present research was designed to study the influence of the conceptus on endocrine and physiological dynamics during the periparturient period in cattle. Selected responses of conceptus and maternal origins were measured during the periparturient period and were related to prepartum interactions between conceptus and maternal units and to postpartum reproductive function.

The bovine uterus was identified as the primary source of F series prostaglandins during the early postpartum period and caruncular tissue was a very active site of synthesis and metabolism of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Measurements of peripheral plasma concentrations of 15-keto-13,14-dihydro-$PGF_{2\alpha}$ (PGFM) were a reliable index of the high degree of uterine synthesis, metabolism and production of $PGF_{2\alpha}$ early postpartum, at a time when uterine blood flow decreased rapidly.

Early postpartum suppression of prostaglandins synthesis did not alter uterine involution but reduced ovarian activity. Replacement therapy with physiological infusions of $PGF_{2\alpha}$ enhanced ovarian activity of ovaries ipsilateral to the previously gravid uterine horn and was associated with greater progesterone release for the first 60 days postpartum.

Maternal peripheral concentrations of endocrine responses of conceptus (prepartum estrogens: estrone, estradiol and estrone sulfate) and maternal (prepartum progesterone and postpartum PGFM) origins were lower in Holstein heifers bred to Angus bulls (H-A) than in those bred to either Holstein (H-H) or Brahman (H-B) bulls and were associated with a lower calf birth weight in H-A. It was hypothesized that measurements in maternal peripheral circulation, of prepartum estrone sulfate and postpartum PGFM concentrations were functional indices of fetal (cotyledon) and maternal (caruncle) parts of the placentome, respectively.

Alterations of functional components of conceptus and maternal unit caused by different conceptus genotype were associated with differential ovarian response to a postpartum GnRH challenge. Higher postpartum PGFM concentrations in H-H and H-B than H-A groups were associated with a faster rate of uterine involution. By affecting the endocrine balance of the maternal unit during the periparturient period in cattle, the sire of fetus, via the conceptus may potentially influence reproductive function and production traits of the dam.

CHAPTER I
INTRODUCTION

Species survival involves adaptation of specific reproductive functions to the environment in which embryonic or fetal development proceeds. In this respect, involvement of the maternal unit in the process of fetal development varies among animals. In lower vertebrates, role of the maternal unit is limited during embryonic development. All nutrient support is contained in the yolk within the shell, but the amniote egg is at the complete mercy of the external environment for gaseous exchange and protection. In contrast, viviparity allows eutherian mammals to exert some control over the environment in which the embryo (or fetus) grows. Adequate nutrient supply and gaseous exchange is assured by the placenta through a constant interaction between the fetus and the maternal unit.

The burden of pregnancy in eutherian mammals imposes a substantial cost to the maternal unit since development of the fetus is associated with growth of fetal membranes, gravid uterus and mammary gland. While the maternal unit provides the uterine environment for continued development of the fetus, the latter, in turn, regulates the maternal unit by influencing the partitioning and mobilization of maternal nutrients for its own development. This induces striking adaptations of the maternal environment by means of homeorhetic signals (Bauman and Currie, 1980) from the developing fetus.

Influence of the conceptus (embryo or fetus, fetal membranes and fluids) on the maternal unit has been recognized throughout gestation, but conceptus maternal interaction is perhaps most evident during the periparturient period. At this time, the fully mature fetus initiates its own release from the uterine environment and, through lactation, the dam continues to supply the juvenile's food. Following this reproductive effort, the dam, which is in a period of infertility, gradually reestablishes an environment that is proper to initiation of a new pregnancy.

Historically in cattle, influence of the growing fetus on the maternal unit was noticed on traits such as timing of parturition, growth of the mammary gland and initiation of lactation. More recently, study of large herd data set indicated that the sire of fetus can account for a portion of the variability in calving ease, milk production and postpartum reproductive function of the dam (Thatcher et al., 1980). Such observations led to the hypothesis that agents of conceptus origin may alter the endocrine environment of the dam. Experimental manipulations of fetal genotype (Eley et al., 1981a; 1981b) and of prepartum environments (Collier et al., 1982; Lewis et al., 1984) had appreciable effect on the endocrine and physiological balance of the maternal unit. Therefore, conceptus-maternal interaction may influence the maternal unit during gestation and this effect may be carried over into the postpartum period.

In the present study, specific responses of the maternal unit were characterized during the prepartum and postpartum period of cattle. The extent to which these responses reflect conceptus-maternal interaction and their potential implication upon reproductive function of the dam are discussed in this dissertation.

CHAPTER II
REVIEW OF LITERATURE

Introduction

The fertilized egg enters the uterus at the late morula stage. At this time, the blastocyst, which contains its own nutrients, consists of an outer layer of trophoblast cells, an inner cell mass and a central cavity or blastocoele. Distinct fate awaits the two cellular components of the blastocyst. The inner cell mass gives rise to the embryo, amnion, yolk sac and allantois while trophoblast cells form the chorion (Steven and Morriss, 1975; Perry, 1981). The whole of the product of conception, i.e., the embryo (or fetus) and its membranes plus fluids, constitutes the conceptus.

At an early stage of development, the conceptus establishes physical contact with the maternal unit, and an organ of exchange between embryo and mother is formed. From this point, survival of the conceptus is assured by maternal nutrients supplied by the chorioallantoic circulation of the true placenta. Bovine conceptus development and evidence of its constant interaction with the maternal unit during pregnancy will be presented. Information concerning early conceptus development, prior to placental formation, can be obtained from Mossman (1937), Amoroso (1952), Steven and Morriss (1975) and Perry (1981).

Bovine Conceptus and Its Uterine Environment

Characterization of bovine conceptus development (Hammond, 1927; Ferrell et al., 1976; Eley et al., 1978; 1979) indicated that growth of the placenta was faster than that of the fetus in early pregnancy, but during later development growth of the fetus far exceeded growth of the placenta. Prior to 100 days, increases of allantoic fluid volume and chorioallantoic membrane weight preceded those of amniotic fluid volume and weights of amniotic membrane and fetus. However, after this time, growth rate of the fetus increased steadily; by 7 months of pregnancy, fetal weight exceeded that of other components of the conceptus (Hammond, 1927; Ferrell et al., 1976; Eley et al., 1978). Ferrell et al. (1976) reported that fetal weight represented 19.2, 36.8, 56.8 and 57.5% of gravid uterine (fetus, fetal fluid, fetal membrane and uterus) weight at 134, 189, 234 and 264 days of gestation, respectively. Eley et al. (1978) estimated that at approximately 100 days of gestation, fetal development proceeded at a rate of approximately 30 g/day. Subsequently, fetal growth rate increased steadily, reached a maximum rate of 220 g/day at approximately day 230 of gestation, and then decreased to less than 100 g/day near term (day 280). Thus, establishment of a placenta, which serves as an organ of exchange between the fetus and the maternal unit, is a prerequisite of fetal growth.

Steven (1975) reviewed various systems for classifying chorioallantoic placentae of different species. Placentae can be classified by shape, relative to the final distribution of chorionic villi over the endometrial surface. In the cow, as in the majority of ruminants, chorionic villi are restricted to circular or oval areas of the chorionic sac (fetal cotyledon) and are separated by relatively smooth chorion. Fetal cotyledons develop only in those parts of the chorion which overlie specialized areas of the endometrial surface of the uterus known as caruncles (Amoroso, 1952; Steven, 1975). Amoroso (1952) described caruncles as specialized nonglandular and well vascularized areas that are essentially local thickenings of subepithelial connective tissue. Fetal cotyledons and uterine caruncles form placental units known as placentomes which are the anatomical structure characterizing the cotyledonary placenta of the cow. Villi emerge as elongated ridges in the cotyledonary areas of the chorion and gradually come to interdigitate with depressions or crypts in the caruncular surface (Amoroso, 1952). Intervillous digitation, known as maternal septa, are formed as fetal villi grow and increase in length (Carter, 1975). Gas exchange and supply of nutrients (small molecules) from maternal to conceptus units occurs through interlocking microvilli within the placentome (Silver and Steven, 1975).

Amoroso (1952) observed that chorionic villi are absent in the intercotyledonary region. In this area, chorion is lined with the same type of epithelium as on the surface of the villi, but in many places, circular and richly vascularized areolae face uterine gland openings. It is believed that areolae are sites for absorption of uterine glandular secretions or histotroph (Bazer and First, 1983).

Formation of the placenta involves apposition of the vascularized chorioallantoic membrane and uterine surface (Amoroso, 1952). Eley et al. (1978) suggested that accumulation of allantoic fluid during the early stages of pregnancy, would favor establishment of the bovine placenta, by forcing the chorioallantoic membrane into apposition with the endometrium. Until day 100 of gestation, increase in allantoic fluid volume exceeded that in amniotic fluid volume. Thereafter, total volume of fetal fluid (amniotic and allantoic) increased with a decrease in accumulation between the fifth and the seventh month (Eley et al., 1978). An additional increase in total fetal fluid volume during the last 2 months of pregnancy was attributed to rapid accumulation of allantoic fluid (Arthur, 1969).

Melton et al. (1951) reported that attachment of the bovine conceptus to the uterine wall was a gradual process with the first point of attachment occurring immediately around the embryo by day 30. Although this process was accompanied initially by gradual erosion of the uterine epithelium (Melton et al., 1951; Amoroso, 1952), regeneration of this tissue layer occurred based on studies with the electron microscope (Bjorkman, 1969; King et al., 1979). Thus, the cotyledonary placenta of the cow is epitheliochoriol in nature. The chorionic epithelium being in close apposition with maternal uterine epithelium, via interlocking microvilli, within the placentome.

Growth of caruncles was stimulated by their contact with the chorion (Hammond, 1927; Amoroso, 1952). King et al. (1980) reported that close apposition of the chorion with caruncular epithelium occurred by day 21 after mating in the cow, and placentome structures were recognizable by day 29. Formation of fetal villi and maternal crypts was initiated by day 33 of gestation, and their development appeared to be reciprocal (King et al., 1979). Firm attachment between fetal cotyledon and maternal caruncle was complete by day 90 (Hammond, 1927) or day 120 (Hutchinson, 1962) of pregnancy. At these times, easy separation of fetal and maternal tissues within the placentome was not possible and resulted in tearing of tissues.

There are few studies describing placentome development throughout gestation. Hammond (1927) reported that the average weight of caruncles in the gravid uterine horn increased from 0.2 g to 34.4 g during the first 5 months of pregnancy and was more than 200 g by 9 months. Caruncular development was much slower in the nongravid uterine horn and was not initiated until pressure was exerted on the caruncles by the turgid membranes. Bjorkman (1969) studied cellular alterations of the bovine placentome and concluded that rapid cell turnover, confined mainly to the cryptal cells, was an indication of continuous growth and remodelling of the bovine placentome during gestation. Hutchinson (1962) reported that bovine caruncles appear to attain their maximum size and vascularity by day 240 of pregnancy.

Marked structural changes occurred in other tissues of the gravid uterus to accomodate the product of conception. Weight of the bovine uterus increased steadily during gestation (Hammond, 1927; Ferrell et al., 1976). Hammond (1927) found that the myometrium enlarged greatly up to 5 to 6 months of pregnancy. According to Hammond (1927), first stage of uterine growth (myometrium) was characterized by cellular hyperplasia and subsequently by cellular hypertrophy. As uterine size increased, tissue began to stretch, and the uterine wall became thinner. Hutchinson (1962) detected evidence of uterine hyperplasia up to day 60 of pregnancy, and definite uterine hypertrophy was observed between days 60 and 181. Uterine stretching was the primary mean of uterine accommodation during the last trimester of pregnancy.

During the second and third months of pregnancy, intercaruncular endometrium developed folds which increased as gestation advanced (Hammond, 1927). Uterine glands in these areas secreted uterine milk, and they became very large in size as uterine tissue grew.

Vascular changes occurred in the bovine uterus throughout pregnancy. Prior to attachment of the bovine conceptus to the uterine wall, Amoroso (1952) observed an increased vascularity which accompanied edema and glandular hypertrophy of the uterine endometrium. Hafez (1974) reported that the main uterine artery ipsilateral to the developing fetus enlarged progressively during gestation. Vascular changes within the gravid uterus were described by Hutchinson (1962). He injected contrast medium into the arterial system of bovine uteri at known gestational ages. There was a progressive development of vascular tissue both in size and number up to day 90 of gestation. After this time, vascular changes of the uterus were characterized primarily by an increase in size of vessels. However, Hutchinson (1962) noticed that vascularization of caruncles followed a different progression in that both number and size of the caruncular vessels increased after 120 days of gestation. During this period, caruncles underwent rapid development, and maximal size and vascularity were attained by day 240 of gestation. In sheep, a species bearing the same type of placenta as the cow, Barcroft and Barron (1946) indicated that there was an increased vascularity of caruncles during late gestation even though weight of the whole placentome was declining.

From the foregoing, it becomes obvious that the developing conceptus induces dramatic changes of the maternal uterus to satisfy its needs. Although a clear understanding of the mechanisms governing conceptus growth and related changes of the uterus is missing, there is evidence that these processes are brought about through constant interactions between conceptus and maternal units. Origin and dynamic changes of selected products produced by conceptus and maternal units during pregnancy will be presented since they may be exerting or associated with major regulatory control of conceptus development.

Endocrine Profiles During Pregnancy and the Periparturient Period

Under normal circumstances, the fetus develops in utero until capable of an independent existence outside the uterus. Both conceptus and maternal endocrine factors are important to maintain pregnancy and to ultimately terminate it. This section intends to familiarize the reader with origin and dynamic changes of selected products during pregnancy and the periparturient period. Information will be presented primarily in relation to progesterone, estrogens and prostaglandins. This does not minimize the importance of other known factors that have been characterized throughout pregnancy. However, a description of the latter is beyond the scope of this review. To complement information concerning the bovine species, data available in ovine and caprine species will be presented, but species differences will be stressed. Part of the information given below was obtained from reviews by Allen (1975), Thorburn et al. (1977), Challis et al. (1977), Nathanielsz (1978), Thorburn and Challis (1977), Hoffmann et al. (1979) and Bazer and First (1983).

Progesterone

Progesterone is essential for maintenance of pregnancy in all species (Thorburn, 1979). Presence of the bovine conceptus at 15 to 17 days initiates the signal that allows for maintenance of the corpus luteum (CL) and continued progesterone production by the CL (Northey and French, 1980). For a comprehensive review of the mechanisms involved in maternal recognition of pregnancy in different species, the reader is referred to a review by Bazer and First (1983).

Several studies indicated that peripheral concentrations of progesterone in cow plasma increased to 8-10 ng/ml between the time of maternal recognition of pregnancy (day 14-16) and day 40 and were maintained at this concentration until day 240 of gestation (Randel and Erb, 1971; Henricks et al., 1972; Scham et al., 1972). During the last month of pregnancy, peripheral concentrations of progesterone declined progressively and fell precipitously over the last two days prior to parturition (Smith et al., 1973; Hoffmann et al., 1973; Fairclough et al., 1975; Eley et al., 1981a; Collier et al., 1982).

Throughout pregnancy, the bovine CL contained large quantities of progesterone and 20_{β}-hydroxy-Δ^4-pregnene-3-one (Gomes and Erb, 1965; Erb, 1977). Evidence suggests that the CL remains functional throughout pregnancy (Erb et al., 1968) and is the primary source of progesterone during this period in the cow (McDonald et al., 1954; Gomes and Erb, 1965; Erb et al., 1971; Thorburn et al., 1977). Ovariectomy caused a drastic decrease in plasma progesterone concentrations and inevitably led to abortion of the fetus if performed before day 200 of gestation (Estergreen et al., 1967; Tanabe et al., 1968). However, if ovariectomy was performed after day 200, pregnancy was maintained up to day 270 in a majority of cows. However, dystocia occurred at delivery and fetal membranes were retained (Estergreen et al., 1967; Chew et al., 1977).

Administration of exogenous progesterone allowed for maintenance of pregnancy in heifers after CL removal, but minimum dose of progesterone required varied with stage of gestation (Tanabe et al., 1968). Exogenous progesterone requirement for pregnancy maintenance decreased progressively from 2.5 mg (per kg of body weight per day) on day 30 of gestation, to zero around day 200, and then increased gradually thereafter to greater than 0.55 mg at day 270. Endogenous plasma concentrations of progesterone sufficient to maintain pregnancy in cows that were ovariectomized after day 200 of gestation were between 1.0 to 1.5 ng/ml or about 10% of the level measured in intact cows (Chew et al., 1979). These concentrations were associated with extraovarian production.

Maternal adrenals appear to be the main source of extraovarian progesterone during pregnancy in cattle. However, the placenta as well as the fetal gonads or adrenals are also potential sources. Balfour et al. (1957) measured concentrations of progesterone that were 10 to 100 times higher in maternal adrenal vein than the corresponding adrenal artery in a pregnant cow. Recently, Wendorf et al. (1983) showed that pregnancy was terminated within 4 days and that plasma progesterone concentrations fell abruptly to a nondetectable level in cows ovariectomized and adrenalectomized on day 215 of gestation. In cows ovariectomized only, pregnancy was maintained and concentrations of progesterone varied between 2 and 4 ng/ml.

Since it displayed minor capabilities to synthesize progesterone in vitro (Ainsworth and Ryan, 1967), bovine placental tissue was considered a potential source of extraovarian progesterone. However, results of in vivo concentration gradients across the bovine uterus

(Wagner et al., 1974; Comline et al., 1974; Hoffmann et al., 1976; Ferrell and Ford, 1980; Ferrell et al., 1983) suggested that there was no net production of progesterone by the gravid uterus. On the contrary, uptake of progesterone by the gravid uterus was observed. This was indicated by higher progesterone concentrations in the jugular vein or uterine artery than in the uterine vein between 165 and 175 days of gestation (Ferrell et al., 1983) or near term (Comline et al., 1974; Wagner et al., 1974; Evans and Wagner, 1976; Hoffmann et al., 1976).

Despite removal of progesterone from maternal systemic blood, concentrations of progesterone in the fetal circulation (0.2 ng/ml) were appreciably lower than in the maternal circulation (Challis et al., 1974; Hoffmann et al., 1976; Hunter et al., 1977; Ferrell et al., 1983). These authors concluded that no apparent transfer of progesterone occurred from the maternal to the fetal unit through the bovine placenta. However, Eley et al. (1979) reported that the amount of total progesterone in allantoic and amniotic fluids rose with fluid volume and suggested that progesterone, presumably of luteal origin, may be sequestered in fetal fluids. Nevertheless, low concentrations of circulating progesterone in the bovine fetus indicated that fetal contribution to the production of progesterone was only minor (Hunter et al., 1977). Thus, production of progesterone throughout pregnancy in the cow is assured primarily by the CL. Other species, such as the goat, the rat and the rabbit, are also CL-dependent throughout pregnancy for production of progesterone.

In this respect, these species are different from sheep whose production of progesterone is assured by the placenta during the last two thirds of pregnancy. Indeed, progesterone production was derived from the CL during the first 50 days of pregnancy (Denamur and Martinet, 1955) but was shifted to the placenta thereafter (Linzell and Heap, 1968) when luteal function was declining (Edgar and Ronaldson, 1958). As presented later in this review, differences exist between CL-dependent and placental-dependent species relative to the synthesis of estrogens and the mechanism leading to the process of parturition.

Luteinizing hormone (LH) from the pituitary gland was the major luteotrophic factor during the estrous cycle in cattle (Hansel, 1967) and was considered as providing the stimulus for CL maintenance during pregnancy (Heap et al., 1973). Estimates of LH concentrations during the last month of gestation in cows were 1.0 to 1.5 ng/ml (Hoffmann et al., 1973; Eley et al., 1981a). LH concentrations remained fairly constant throughout this period and luteolysis was not associated with any acute change in concentrations of this hormone.

A report by Anderson et al. (1979) indicated that luteal support in cattle does not depend only on LH since pregnancy was maintained and the CL remained functional after hypophyseal stalk transection in midpregnancy. Furthermore, there was little evidence of a luteotrophic role for prolactin in the cow (Karg and Schams, 1974; Hansel et al., 1975). Thus, functional luteotrophic support may be derived from a source other than the pituitary gland during the second half of gestation.

Luteotrophic support during pregnancy has been studied more intensively in the goat, also a CL-dependent species for progesterone production. Hypophysectomy at any stage of pregnancy led to abortion 4 to 9 days later, and LH was essential for luteal maintenance (Buttle, 1978). However, Thorburn and Schneider (1972) proposed that a placental hormone may provide additional stimulation of the CL around midpregnancy. Consistent with this view, Currie and Thorburn (1974) found that following hysterectomy of the goat between day 30 to 50 of pregnancy progesterone concentrations decreased from 5 to 7 ng/ml to about 2 ng/ml and remained at this level until 147 to 184 days. A caprine placental lactogen was isolated (Buttle et al., 1972), and increasing concentrations were measured in maternal peripheral plasma of the pregnant goat between days 50 and 130 of gestation (Currie et al., 1977). Collectively, these results introduced the possibility that a placental luteotrophin (perhaps placental lactogen) may be essential for full function of the CL in this species.

In cattle, two compounds of placental origin and with potential luteotrophic capability have been identified. Bolander et al. (1976) reported that concentrations of bovine placental lactogen were maintained at a relatively high level ($\approx$ 875 ng/ml) from day 160 of gestation until a few days prepartum. A temporal relationship of the decline in bovine placental lactogen and that of progesterone was interesting in that it suggested that a placental lactogen may have a luteotrophic function in cattle. However, Schellenberg and Friesen (1981) indicated that bovine placental lactogen was virtually absent from the maternal circulation when cross-reactivity with prolactin was accounted for in their assay. Consequently, bovine placental lactogen may function locally to influence fetal well-being. Bovine placental lactogen has been isolated from either extracts of cotyledonary tissue or culture media following incubations with cotyledonary tissue (Kensinger, 1982; Arima and Bremel, 1982). Potential role of bovine placental lactogen as a luteotrophin remains unknown at the present time.

Ailenberg and Shemesh (1983) purified a chorionic gonadotropin-like protein from bovine cotyledons between 50 and 100 days of pregnancy. This substance inhibited binding of labeled human chorionic gonadotrophin (HCG) in membranes of rat testicular tissue and enhanced steroidogenesis by bovine granulosa cells and rat leydig cells. These authors suggested that a chorionic gonadotropin-like protein may be involved in prolonging life span of the CL during pregnancy in cattle. Thus, the possibility exists that products of conceptus origin influence the production of progesterone by the maternal CL.

Estrogens

Development of the bovine conceptus is associated with changes in estrogen concentrations in fetal fluids and maternal plasma. Eley et al. (1979) reported that estrone and estrone sulfate in maternal peripheral plasma were not detected until day 70 of gestation. Between days 70 and 111, plasma estrone sulfate concentrations increased from 140 to 581 pg/ml. Robertson and King (1979) reported that concentrations of estrone sulfate in peripheral plasma continued to increase until day 147 and then remained elevated at concentrations varying between 1100 and 3500 pg/ml until near term. Estrone concentrations paralleled those of estrone sulfate but at concentrations that were approximately 3% of estrone sulfate (Tsang et al., 1975; Robertson and King, 1979; Hoffmann et al., 1979; Eley et al., 1979; 1981a). During the last 2 to 3 weeks of gestation, concentrations of conjugated and unconjugated estrogens increased appreciably and then fell precipitously to almost undetectable levels following delivery of fetus and placenta (Robinson et al., 1971; Smith et al., 1973; Comline et al., 1974; Eley et al., 1981a; Collier et al., 1982).

Concentrations of estrogens in fetal fluids were characterized by Eley et al. (1979) and Robertson and King (1979). An initial increase in estrone and estrone sulfate in allantoic fluid was apparent on day 40 (Eley et al., 1979). Between days 33 and 111 of gestation, concentrations of estrone increased from 2 to 144 pg/ml in allantoic fluid and paralleled those of estrone sulfate which increased from 0.21 to 220 ng/ml (Eley et al., 1979). Concentrations of estrone sulfate in allantoic fluid reached a peak concentration of 475 ng/ml around day 133, dropped precipitously until day 170 and then underwent a second increase of lower magnitude (200 ng/ml) between days 200 and 250. Thereafter and until term, concentrations of estrone sulfate in allantoic fluid decreased progressively (Robertson and King, 1979). Fluctuations that were similar to those observed by Robertson and King (1979) for allantoic fluid concentrations of conjugated estrogens during mid-pregnancy also were observed for free estrone and estradiol-17_β in plasma of the uterine vein (Ferrell and Ford, 1980). In both studies, it was suggested that synthesis of estrogens may be decreased for a period of time during mid-gestation, and after day 200, synthesis of estrogen may arise from a new source within the gravid uterus.

The initial rise in estrogen (estrone and estrone sulfate) in allantoic fluid at day 40 was presumably of conceptus origin and was associated with the early increase in placental membrane weight (Eley et al., 1979; Robertson and King, 1979). At this time (day 40), the allantoic membrane was fully expanded, vascularization of the chorionic surface was secured, and intimate contact with the maternal unit had been established due to placentome formation (Melton et al., 1951; Chang, 1952; King et al., 1979). Higher concentrations of free estrogens in allantoic than amniotic fluid during the first trimester of gestation were indicative of estrogen production by the chorio-allantoic membrane (Eley et al., 1979). These authors also observed a higher concentration of estrone sulfate in amniotic than allantoic fluid. It was suggested that conjugation of free estrone may occur in the fetus and that the conjugate may be pooled in the amniotic fluid. This would minimize activity of estrogens that are available for transfer to the maternal peripheral plasma.

Catheterization of uterine, umbilical and fetal vasculature provided insight on the site of production of estrogens during the second half of pregnancy in cattle. Higher concentrations of unconjugated estrogens in the uterine vein than in maternal peripheral blood suggested synthesis of estrogens by tissue within the gravid uterus (Comline et al., 1974; Peterson et al., 1975; Hoffmann et al., 1976; Ferrell and Ford, 1980; Ferrell et al., 1983). The placenta likely is to be a source of estrogen production since it contains an active aromatase system (Ainsworth and Ryan, 1966). Results by Hoffmann et al. (1976) and Ferrell et al. (1983) are supportive of placental rather than fetal production of estrogens. It was reported in these two studies that concentrations of estrogens (conjugated and/or free) were greater in plasma of placental effluent (umbilical vein or fetal vena cava) than in that of placental influx (umbilical artery or fetal femoral artery). Experiments of in vitro perifusions and in vivo perfusions (Hoffmann et al., 1979) provided evidence that the fetal part of the placentome (cotyledon) is the major source of estrogens during late gestation in cattle. Estrone was the major estrogen produced within the fetal cotyledon and a significant portion was conjugated directly at the site of synthesis.

Indeed, conjugated forms of estrogens predominated largely over their corresponding free forms in both maternal (Tsang et al., 1975; Hoffmann et al., 1976; 1977; Eley et al., 1979; 1981a; Robertson and King, 1979) and fetal plasma (Hoffmann et al., 1976). However, qualitative and quantitative differences in estrogen exist between fetal and maternal circulations in the cow. Hoffmann et al. (1979) concluded that upon its release into the fetal circulation, estrone was reduced into estradiol-17_{α}, whereas estrone remained the primary estrogen in maternal circulation. Although estradiol-17_{α} was not measured, concentrations of free estradiol-17_{α} that averaged 20 to 25 pg/ml in the fetal circulation were measured and were reported as being higher than those of free estrone (Peterson et al., 1975; Ferrell et al., 1983). In addition, prepartum increases in concentrations of free and conjugated estrogens in maternal blood were not accompanied by similar changes in fetal blood (Peterson et al., 1975; Hoffmann et al., 1977). Thus, as suggested by Hoffmann et al. (1976) and Ferrell et al. (1983), extensive synthesis of estrogens occurs within the placenta (fetal cotyledon; Hoffmann et al., 1979), but this organ appears to be a selective barrier to steroid hormones. It seems to regulate the type and amount of estrogens secreted into fetal and maternal compartments.

The relative importance of estrogens in the process of parturition in different species (to be discussed later) stimulated research on their mode of synthesis during the prepartum period. In several species, increases in concentrations of cortisol in fetal circulation were associated temporarily with prepartum increases in estrogen concentrations in maternal plasma (see reviews by Thorburn et al., 1977; Challis et al., 1977; Bazer et al., 1983). However, although placental production of estrogens is a common feature among species, differences exist relative to the origin of estrogen precursors.

In sheep, a species that relies on placental production of progesterone for maintenance of pregnancy, fetal cortisol enhanced enzymatic activities of placental 17_{α}-hydroxylase (Anderson et al., 1975), C-17, 20 lyase (Steele et al., 1976) and aromatase (Mann et al., 1975). This caused the placenta to produce predominantly estrogens rather than progesterone. Placental C_{21} substrates such as progesterone (Anderson et al., 1975) or pregnenolone (Nathanielsz et al., 1982) were converted into C_{19} androgens (androstenedione; Steele et al., 1976) which are then aromatized to C_{18} estrogens.

In primates, which are dependent on the placenta for progesterone production, Diczfalusy and Mancuso (1969) introduced the concept of the "feto-placental unit" for production of estrogens. This stems from the fact that in vitro studies of placental steroid biosynthesis demonstrated aromatase activity but a deficiency of 17_{α}-hydroxylase and C-17,20 lyase (Challis et al., 1977). The latter two enzymes being necessary for conversion of C_{21} steroids to C_{19} androgens. Thus, as the primate placenta cannot synthesize androgens, these must be supplied by another tissue. Diczfalusy and Mancuso (1969) proposed a model whereby C_{21} steroids (progesterone) of placental origin are transported to fetal adrenals to be converted into C_{19} androgens.

Thereafter, androgens returned to the placenta where they are aromatized into C_{18} estrogens.

In contrast, it appears that C_{19} androgens derived from fetal adrenal glands are unlikely to be major estrogenic precursors prepartum for cattle and goats. Flint et al. (1978) infused dexamethasone into fetal goats and found that although fetal adrenal activity was suppressed as judged by low fetal cortisol concentrations, maternal estrogen levels were still increasing. In the cow, placental production of estrogens was still maintained after fetectomy which led Hoffmann et al. (1979) to suggest that precursors (androgen) for placental production of estrogens originates in maternal plasma and that, as in the goat, the concept of a "feto-placental unit" was not applicable.

The nature of precursors for synthesis of estrogens by the bovine placenta remains obscure. Early studies in vitro indicated that bovine placental tissue may synthesize estrogens from C_{19} (androgens) but not from C_{21} steroid precursors (Ainsworth and Ryan, 1966; Hoffmann et al., 1979). More recently, however, Evans and Wagner (1981) reported that the bovine placenta has the capability in vitro to produce estrogens from C_{21} (17_{α}-hydroxyprogesterone and pregnenolone) precursors and that induction of enzymes for converting pregnenolone or related C_{21} substrates to estrogens may be involved in the prepartum rise in estrogens. Eley et al. (1981a) observed an inverse relationship between prepartum concentrations of estrogens and progesterone. They proposed that progesterone of luteal origin or its metabolites may be precursors for synthesis of estrogens by the bovine placenta.

A controversy still exists as to whether progesterone of luteal origin is related to placental production of estrogens in cattle. In goats, activity of placental 17_α-hydroxylase increased prior to parturition (Flint et al., 1978), and mid-pregnancy placentae differed from those obtained at term with respect to their ability to metabolize progesterone (Flint et al., 1979). Exposure to either endogenous or administered glucocorticoids resulted in the formation, in vitro, of 17_α-hydroxylated progesterone metabolites. From these observations, Flint et al. (1978; 1979) proposed that, as in sheep, activations of placental enzymes near parturition in goats may lead to increased placental production of estrogens from C_{21} precursors of maternal origin. Thorburn (1979) indicated that progesterone of CL origin may be taken up by the placenta and converted to estrogens during the second half of pregnancy in goats. Similarities exist between cattle and goat relative to metabolism of progesterone by mid-term and term placentae and basic endocrine functions (Flint et al., 1979). Whether these similarities can be extended to placental synthesis of estrogens from precursors of CL origin in cattle remains a matter of debate. Since concentrations of estrogens still increased near parturition in ovariectomized cows, Hoffmann et al. (1979) refuted the view that estrogen production depends on precursors supplied by the ovary and that the inverse changes in estrogens and progesterone were related. On the other hand, Chew et al. (1979) reported that although all estrogens measured in maternal plasma increased prepartum, they were appreciably lower in ovariectomized than intact cows.

Prostaglandins

Appreciably more prostaglandin $F_{2\alpha}$($PGF_{2\alpha}$) was measured in uterine secretions at day 16 and 19 of pregnancy than during corresponding days of the bovine estrous cycle (Bartol et al., 1981). This probably is associated with the extensive production of $PGF_{2\alpha}$ and PGE_2 by day 16 and 19 bovine conceptuses (Lewis et al., 1982). Between day 33 and 111 of gestation in cattle, Eley et al. (1979) detected significant amounts of $PGF_{2\alpha}$ in fetal fluids. These authors indicated that higher concentrations of $PGF_{2\alpha}$ in allantoic than amniotic fluids (14.8 vs 3.5 ng/ml) suggest a uterine origin of $PGF_{2\alpha}$. Possibly, $PGF_{2\alpha}$ production occurred at the site of placental attachment. Subsequent transport of $PGF_{2\alpha}$ through the chorioallantoic membrane may have led to a sequestration of $PGF_{2\alpha}$ in the allantoic fluid.

Concentrations of various prostaglandins [$PGF_{2\alpha}$; 15-keto-13,14-dihydro-$PGF_{2\alpha}$ (PGFM); PGE_2; 6-keto-prostaglandin F_1 ; thromboxane B_2] were low (< 275 pg/ml) in uterine and umbilical vessels (vein and artery) between days 80 and 260 of pregnancy in cattle (Gimenez et al., 1983). During this period, no gestational trend was evident and venous-arterial differences in concentrations of $PGF_{2\alpha}$ and PGFM across the uterus were approximately 125 pg/ml. Fairclough et al. (1975) reported that concentrations of $PGF_{2\alpha}$ in utero-ovarian vein remained constant at 125 to 150 pg/ml until 5-7 days before term when the concentrations rose gradually to a maximum of 5 to 9 ng/ml at delivery.

Due to rapid metabolism of $PGF_{2\alpha}$ in the lung (Ferreira and Vane, 1967; Davis et al., 1980; Maule Walker and Peaker, 1981), concentrations of $PGF_{2\alpha}$ in peripheral circulation were undetectable during the estrous cycle (Knickerbocker et al., 1984) or pregnancy (Eley et al., 1979) in cattle. Measurement of PGFM, the primary metabolite of $PGF_{2\alpha}$ (Kindahl et al., 1976), has provided an alternative method for estimating the secretion of $PGF_{2\alpha}$ by uterine tissue in cyclic cows (Knickerbocker et al., 1984). This technique has been used in periparturient cattle to assess levels of $PGF_{2\alpha}$. However, one must recognize that measurements of PGFM in peripheral circulation of periparturient cattle have not yet been shown to account for uterine production of $PGF_{2\alpha}$. Nevertheless, peripheral concentrations of PGFM in cattle increased progressively during the last week of pregnancy, reached peak values on days 2 or 3 postpartum and then decreased progressively to a basal concentration by day 15 (Edqvist et al., 1978; Eley et al., 1981b; Lindell et al., 1982; Lewis et al., 1984).

The prepartum rise in F series prostaglandins ($PGF_{2\alpha}$ and PGFM) is a common feature among various species (see reviews by Thorburn et al., 1977; Nathanielsz, 1978; Thorburn and Challis, 1979) and has been implicated in the parturition process (to be discussed later). However, persistance of high levels of PGFM during the postpartum period appears to be unique to cattle and sheep (Lewis and Bolt, 1983). In horses (Allen and Pashen, 1981) and pigs (Nara and First, 1981), concentrations of PGFM peaked at parturition and returned to basal level within 24 h. Thus, in ruminants (cattle and sheep), the postpartum increase in PGFM appears to be a response of the maternal unit expressed only after the feto-placental unit has been expelled.

The source of F series prostaglandins during the periparturient period in cattle is still unkwown since only peripheral PGFM concentrations (Edqvist et al., 1978; Eley et al., 1981b; Lindell et al., 1982; Lewis et al., 1984) or uterine venous concentrations of $PGF_{2\alpha}$ (Fairclough et al., 1975) were measured. In goat, Maule Walker and Peaker (1981) demonstrated that the mammary gland produces significant amounts of F series prostaglandins postpartum. On the other hand, a uterine source also may be suspected since an increase in PGFM coincided with delivery of the feto-placental unit, and its decrease was associated positively with reduction in size of the gravid uterine horn postpartum (Eley et al., 1981b; Lindell et al., 1982).

Intrauterine tissues (primarily the endometrium) have the ability to produce various prostaglandins during the estrous cycle or early pregnancy in several species (see review by Bazer and First, 1983). Venous-arterial differences in concentrations of prostaglandins ($PGF_{2\alpha}$, PGE_2 and PGFM) across the uterus near term in sheep and goats, suggested that tissues within the uterus were producing prostaglandins (Mitchell et al., 1976; Keirse et al., 1977; Mitchell and Flint, 1978a). In vitro studies of intrauterine tissues collected at parturition in sheep and goat, indicated that PGE_2, $PGF_{2\alpha}$ and PGFM were detectable in myometrium and caruncular endometrium (Keirse et al., 1976; Mitchell and Flint, 1977; 1978b). Furthermore, Evans et al. (1982) reported that 6-keto-$PGF_{1\alpha}$ (the hydrolysis product of prostacyclin) and thromboxane B_2 also were present in fetal and maternal intrauterine tissues of the late pregnant sheep.

Production of prostaglandins in the myometrium was appreciably lower than in the caruncular endometrium in sheep (Mitchell and Flint, 1978b) and goats (Mitchell et al., 1978). Qualitative differences were reported for production of prostaglandins in caruncles and cotyledons. The fetal part of the placentome (cotyledon) was the major site of PGE_2 production in sheep (Mitchell and Flint, 1977) and in cattle (Williams and Gross, 1981). In agreement with the previous observation, PGE_2 was the major prostaglandin found in the circulation of fetal lambs (Challis et al., 1976; Mitchell et al., 1980). Production of F series prostaglandins ($PGF_{2\alpha}$ and PGFM) was higher in caruncles than cotyledons (Mitchell and Flint, 1977).

Synthesis of prostaglandins (prostacyclin and $PGF_{2\alpha}$) was inhibited in maternal caruncles collected on day 260 of gestation but not in those collected at term in cattle (Shemesh et al., 1984). A cytosolic protein that was a potent inhibitor of prostaglandin synthetase was isolated in term bovine fetal cotyledons (Shemesh et al., 1981). Therefore, temporal changes in prostaglandin production may be related to the presence of an endogenous inhibitor (Shemesh et al., 1984). Removal of cotyledonary inhibition at parturition (delivery of calf and fetal placenta) would likely increase production of $PGF_{2\alpha}$ by the maternal caruncle. The tissues producing F series prostaglandins during the early postpartum period in cattle need to be documented to understand its potential physiological roles.

Interrelationships of Conceptus and Maternal Units

There is evidence indicating that the conceptus has the ability to interact with the maternal unit during gestation and parturition. Evidence of this interaction and potential role of selected hormones during pregnancy and parturition will be presented.

Gestation

In various species, the conceptus signals its presence to the maternal unit early in pregnancy (see review by Bazer and First, 1983). In cattle, induction of CL maintenance during pregnancy was associated with presence of a conceptus between days 15 and 17 post-mating (Northey and French, 1980). At the time of maternal recognition of pregnancy, Ford et al. (1979) demonstrated that the early bovine conceptus exerts a local control on uterine blood flow to possibly assure optimum uterine conditions for its development during this critical period.

Growth of the uterus, in mice, was regulated systemically by hormones of both conceptus and maternal origin, and locally by the physical presence of the conceptus (Carsten, 1968). In ruminants, growth of caruncular and intercaruncular endometrium also was stimulated by physical presence of the conceptus (Hammond, 1927; Amoroso, 1952), but the exact nature of the stimulus (local and/or systemic) remains unclear. Reynolds (1949) suggested that progesterone produced by the corpus luteum in various species stimulated uterine growth. Caruncular growth in pregnant ewes (Alexander and Williams, 1966) and protein secretions in nonpregnant ewes (Moffatt et al., 1980) were stimulated by administration of progesterone. On the other hand, Miller et al. (1983) failed to demonstrate any important role for estrogens or progesterone on caruncular growth. However, the increase in secretory activity of the intercaruncular endometrium around mid-gestation was associated with a marked increase in the level of nuclear progesterone receptors.

When survival of the conceptus becomes dependent upon the maternal unit for supply of nutrients and exchange of blood gases, blood flow to the uterus becomes an important component which may affect this function (see review by Silver and Steven, 1975). The period of attachment and implantation of the bovine conceptus (days 25-30) coincided with a progressive rise in UBF (Ford et al., 1979). Likewise, blood flow to the gravid uterine horn increased dramatically from less than 100 ml/min to approximately 2000 ml/min between 92 and 134 days postmating (Ferrell and Ford, 1980). This was the interval during which rapid fetal growth was initiated (Hammond, 1927; Ferrell et al., 1976; Eley et al., 1978; Ferrell and Ford, 1980). UBF increased further until day 200 of gestation and remained fairly constant at about 3500 ml/min during the last 2.6 months of pregnancy (Ferrell and Ford, 1980).

During the second half of pregnancy in sheep (Greiss and Anderson, 1970) and goats (Cotter et al., 1969), flow of blood per kg of uterus, placenta and fetus remained constant but showed a slight decline in cattle (Ferrell and Ford, 1980). Nevertheless, it appears that blood flow to the uterus (ml/min/kg) during this period increased in relation to fetal growth. In pregnant ewes, Makowski et al. (1968) reported that as the fetus grew, blood flow to placentomes increased continuously whereas flow to the intercaruncular endometrium and myometrium remained constant. During the last month of pregnancy, these authors indicated that 84% of total uterine blood flow was ascribed to placentomes, 13% to intercaruncular endometrium, and 3% to myometrium.

Rankin et al. (1975) provided evidence that the conceptus exerts some control over UBF during pregnancy. In pregnant sheep, ligation of umbilical circulation to several cotyledons caused a reduction in maternal placental blood flow to these cotyledons. Similarly, killing some of the fetuses in pregnant rabbits resulted in a decrease in maternal placental flow only to the dead fetuses. Blood flow to the gravid uterine horn in unilaterally pregnant ewes (ligation of one uterine horn) was higher than that to the nongravid horn and that to the horn ipsilateral to the CL of control animals (Caton et al., 1984). It was suggested that local factors, closely associated with the presence of a conceptus may stimulate UBF.

Evidence has been presented that administration of estrogens causes an increase in UBF in ewes (Huckabee et al., 1970), cows (Roman-Ponce et al., 1978) and sows (Dickson et al., 1969). Increases in UBF during pregnancy in cattle coincided with increases in estrogen concentrations in uterine flushings (Ford et al., 1981), uterine vein (Ferrell and Ford, 1980) and allantoic fluid (Eley et al., 1979; Robertson and King, 1979). These associations indicated that estrogens of conceptus origin may be implicated in control of UBF during pregnancy.

Control of UBF during the estrous cycle and pregnancy in various species was reviewed by Ford (1982). Norepinephrine released from periarterial adrenergic nerves acts on smooth muscle cells via either α-adrenergic receptors to cause vasoconstriction (Greiss and Pick, 1964) or via β-adrenergic receptor to cause vasodilation (Ford and Reynolds, 1981). Ford (1982) suggested that estradiol, after its conversion to a catechol estrogen, reduces activity of α-adrenergic receptor in periarterial nerve thereby causing an increase in UBF. Furthermore, pregnancy appears to be associated with marked reduction in function of periarterial adrenergic nerves that would lead eventually to maximal arterial vasodilation and uterine blood flow during late pregnancy.

There is a growing body of evidence that prostaglandins of conceptus origin also could be implicated in control of UBF (Terragno et al., 1974; Rankin, 1976; Rankin and McLaughlin, 1979; Clark et al., 1982). PGI_2 and PGD_2 increased, while PGE_2 and $PGF_{2\alpha}$ decreased UBF in pregnant sheep (Clark et al., 1982). These authors suggested that PGI_2 and PGD_2 of conceptus origin may be likely candidates in maintaining high levels of UBF.

The main purpose of the conceptus in inducing changes in various functions of the maternal unit is to satisfy the needs of the growing fetus. In this respect, the conceptus acts as a parasitic structure which can grow only by forcing the host to feed it. When this mission has been accomplished by the dam, the mature fetus gives the signal that will initiate its release from the uterine environment.

Parturition

Ability of the conceptus to control gestation length constitutes probably the most documented example of a conceptus and maternal interaction. As early as 1924, fetal sex was noted to influence gestation length in that male fetuses were carried for a longer period than female fetuses in cattle (Fitch et al., 1924). Observations that prolonged pregnancies were related to anencephaly of the fetus in humans (Malpas, 1933), to absence or malfunction of the fetal pituitary and to underdevelopment of fetal adrenal glands in sheep (Binns et al., 1963) and cattle (Holm, 1967) served as a basis to develop an understanding of the mechanism leading to parturition.

The classical experiments of Liggins (1969; 1973) clearly demonstrated that activation of the fetal pituitary-adrenal axis is responsible for initiation of parturition in sheep. Fetal hypophysectomy or adrenalectomy led to a prolongation of pregnancy (Liggins et al., 1967; Drost and Holm, 1968). Following hypophysectomy in fetal sheep, replacement therapy with ACTH caused premature delivery (Jones et al., 1978) suggesting that ACTH is the essential product of pituitary origin to initiate parturition. During normal parturition (Bassett and Thorburn, 1969) or after intra-fetal infusion of ACTH (Bassett and Thorburn, 1973), prepartum concentrations of cortisol in fetal plasma increased reflecting fetal adrenal production of cortisol (Nathanielsz et al., 1972). Premature delivery was induced, in sheep, by intrafetal infusion of a glucocorticoid (Liggins, 1969).

Various experimental approaches that were reviewed by Thorburn et al. (1977), Challis et al. (1977) and Bazer and First (1983) provided strong evidence that signals initiating parturition in goat, swine and cattle also originate in the pituitary adrenal axis of the fetus. In cattle, concentrations of cortisol in intact fetal calf plasma rose from about 5 ng/ml at 20 days before parturition to levels around 70 ng/ml on the day of calving (Comline et al., 1974; Hunter et al., 1977). Parturition occurred within 7 days after corticotropin infusion (Welch et al., 1973; Comline et al., 1974) or within 3 days after infusion of dexamethasone (Comline et al., 1974).

Activation of the fetal pituitary adrenal axis in domestic animals leads to a cascade of endocrine and physiological events that ultimately culminates in delivery of the feto-placental unit. Maintenance of pregnancy and uterine quiescence is associated with high levels of circulating progesterone. In the pregnant rabbit, Csapo (1956) developed the concept that progesterone blocks myometrial activity during pregnancy and that withdrawal of this "block" is a prerequisite for parturition to occur. Lye and Porter (1978) demonstrated that the classical characteristic of the progesterone block could be extended to sheep. Progesterone caused a dose-dependent suppression of the amplitude of myometrial contractions, a reduction in the rate of rise of pressure during intrauterine pressure cycles and a loss of reactivity to oxytocin. In cattle, evidence supports the applicability of the progesterone block hypothesis during pregnancy. Cows exhibiting prolonged gestation in association with fetal abnormalities also maintained high levels of progesterone (Holm and Short, 1962). Likewise, exogenous progesterone given in large amounts had the ability to prevent premature delivery induced by glucocorticoids (Jochle et al., 1972). Thus activation of fetal pituitary adrenal axis must necessarily initiate mechanisms leading to a diminution of progesterone concentrations in the maternal unit.

In sheep, a placental-dependent species for progesterone production, an increase in fetal production of cortisol induced the rate limiting enzymes 17-$_\alpha$ hydroxylase (Anderson et al., 1975), C-17,20 lyase (Steele et al., 1976), and aromatase (Mann et al., 1975). This causes the placenta to secrete estrogens rather than progesterone. In CL-dependent species such as cattle, goats and pigs, parturition also is preceded by a drop in maternal progesterone levels and a rise in maternal estrogens. However, the sequence of events leading to these endocrine changes is not as clear. Increased fetal production of cortisol apparently stimulated estrogen production by the bovine placenta (Comline et al., 1974; Hunter et al., 1977), and maternal concentrations of $PGF_{2\alpha}$ increased in response to estrogens (First et al., 1982).

Exogenous administration of $PGF_{2\alpha}$ was luteolytic in cyclic cows (Hafs et al., 1974; Lauderdale, 1974; Thatcher and Chenault, 1976) and administration of cloprostenol (a prostaglandin analog) caused abortion in cattle (Lindell et al., 1982). Gradual increases in prepartum concentrations of $PGF_{2\alpha}$ in uterine vein (Fairclough et al., 1975; Hunter et al., 1977; First et al., 1982) or of PGFM in the maternal peripheral circulation (Edqvist et al., 1978; Eley et al., 1981b) coincided with the prepartum decline in progesterone. Despite an infrequent blood sampling schedule, that precluded a real assessment of cause and effect relationships, these authors suggested that the prepartum increase in concentrations of F series prostaglandins may lead to final regression of the CL. Hoffmann et al. (1979) questioned this interpretation on the basis that the drop in progesterone concentrations in the uterine vein in cattle preceded the increase in F series prostaglandins. In swine and sheep, experiments involving inhibition of prostaglandin synthesis and replacement with $PGF_{2\alpha}$ indicated that luteolysis depends on elevated $PGF_{2\alpha}$ (Nara and First, 1981).

Central to the process of parturition in various species is the increase in the estrogen:progesterone ratio and F series prostaglandins initiated by a rise in fetal cortisol. Drawing from studies in sheep and laboratory animals, considerable knowledge was gained with respect to the physiological and biochemical changes associated with initiation of coordinated uterine contractions that facilitate parturition. Gap junctions, which are sites of low resistance contact allowing current flow between excitable cells developed rapidly close to term and were believed to favor synchronized and coordinated uterine contractions (Garfield et al., 1977; 1979; 1980). Formation of gap junctions was correlated positively with prepartum changes in estrogen:progesterone ratio in rat and sheep (Garfield et al., 1979; Puri and Garfield, 1982). Likewise, the ability of myometrial tissue to respond to oxytocin was increased prior to parturition. Oxytocin receptors develop in response to the prepartum rise in estrogen:progesterone ratio in rats and guinea pigs (Alexandrova and Soloff, 1980). Existence and hormonal control of gap junctions and oxytocin receptors need to be confirmed in cattle, but Bazer and First (1983) indicated that the pattern of myometrial activity prior to parturition is similar in several species.

Besides its potential role in luteolysis, $PGF_{2\alpha}$ has been implicated in the process of uterine contractions. According to Liggins (1979), prostaglandins stimulate uterine contraction by inducing liberation of Ca^{++} from intracellular binding sites and by causing a slow membrane depolarization that increases the frequency of action potentials. Garfield et al. (1980) reported that indomethacin, a prostaglandin synthetase inhibitor, prevents formation of gap junctions in vitro, and this effect could be overcome by addition of arachidonic acid, a precursor of prostaglandin synthesis. In sheep, inhibition of prostaglandin synthesis interfered with delivery presumably by inhibiting prostaglandin stimulation of uterine contraction. Prostaglandin stimulation may be direct and/or via stimulation of oxytocin release (Mitchell and Flint, 1978a). Oxytocin facilitates uterine contraction by lowering the threshold potential for initiation of action potential activity and has also a direct action on the rate of Ca^{++} influx. Chan (1977) reported that prostaglandins and oxytocin, separately or in concert, can elicit uterine contractions.

Korenman et al. (1977) proposed a model for uterine contractions in which prostaglandins (and other contractile agents) bind to specific receptors at the cell surface and cause a decreased cellular concentration of cyclic AMP (cAMP). A fall in cAMP concentrations results in deactivation of a catalytic subunit of protein kinase, decreased translocation of protein kinase to the cell membrane (sarcoplasmic reticulum) and decreased phosphorylation of specific membrane proteins. This is associated with a rise in intracellular free Ca^{++} which cause formation of a Ca^{++}-calmodulin complex. This complex binds to and activates myosin kinase, which phosphorylates myosin, enabling myosin to interact with actin to cause contraction (see review by Bazer and First, 1983). The extent at which these models are applicable to domestic species has not been tested. Nevertheless, evidence has been presented in this section that the normal process of parturition is dependent upon a close interaction between conceptus and maternal unit.

Experimental Manipulations of Conceptus and Maternal Environments

A uterine environment that is suitable for conceptus development is dependent upon a constant interaction between conceptus and maternal units. Bauman and Currie (1980) defined homeorhesis as a coordinated change in metabolism of body tissues to support a dominant developmental or physiological process. Maternal adaptations to meet fetal needs are most critical and in turn are expected to be regulated by homeorhetic signals from the conceptus (Bauman and Currie, 1980). The nature of the regulatory agents is not clear, but capability of adaptation of conceptus and/or maternal units can be illustrated by observations of physiological and endocrine responses following various alterations of their intimate relationship. Since the number of experiments of this nature is limited in cattle, reference also will be made to other domestic or laboratory species.

Physiological Responses

Sir Joseph Barcroft (1947) proposed that placentae of different age but of equal size represent organs of metabolic exchange with similar physiological characteristics. Based on this assumption, he suggested that early in pregnancy, the ability of the placenta to supply nutrients and O_2 far exceeds the metabolic requirements of the fetus. Then, as the fetus grows, the margin of safety between placental supply and fetal needs would become narrower and may actually reach a point at which fetal growth becomes limited by placental size. In this respect, the conceptus becomes a victim of its environment since it has no control over the availability of oxygen.

However, more recent experiments involving chronic sampling of umbilical and fetal blood on the undisturbed fetus demonstrated that ovine (see review by Barron, 1970) and bovine (Silver et al., 1973) fetuses live in a relatively constant environment with respect to the availability of oxygen. According to Barron (1970), this constancy is achieved through an increase in the functional capacity of the three units of the placenta directly involved in the oxygen supply of the fetus. These are the uteroplacental and umbilical circulations and the tissue interposed between. The present section will attempt to illustrate the dynamic relationship between conceptus and maternal unit relative to fetal growth and survival in stressful situations.

Pregnant sheep maintained at high altitude or under conditions of low ambient oxygen (O_2) showed a decrease in partial pressure of oxygen (PO_2) in maternal blood but fetal PO_2 was scarely different than that observed under normal conditions (Makowski et al., 1968). Adaptations observed during pregnancy under such conditions (high altitude or low ambient PO_2) included an increase in placental weight relative to fetal weight, an increase in maternal blood flow and an increase in placental diffusing capacity (Metcalfe et al., 1967; Kruger and Arias-Stella, 1970; Gilbert et al., 1977).

Partial caruncIectomy of sheep uteri were made to impose limitations on placental development during gestation (Alexander, 1964; Robinson et al., 1979). Fetal and total placental weight, as well as the number of cotyledons, were reduced following surgical removal of caruncles. However, birth weight was more closely related to the weight of the cotyledons than to the number of caruncles removed. Indeed, reduction in number of cotyledons was compensated partly by an increase in weight of individual cotyledons (Alexander, 1964), a larger proportion of heavier cotyledons (> 15 g) and an increased packed cell volume in the fetus (Robinson et al., 1979). Nevertheless, compensation was only partial and analyses of fetal blood responses indicated that fetal growth retardation due to restriction of placental growth was associated with chronic hypoxemia, polycythemia and hypoglycemia (Robinson et al., 1979).

In contrast to the previous approach, total number of available caruncles in sheep was reduced by either surgical removal or ligation of one uterine horn (Cefalo et al., 1977; Bazer et al., 1979; Caton et al., 1984). Normal size fetuses were born and O_2 consumption was normal. Process of adaptation involved a compensatory hypertrophy of the placentomes (Cefalo et al., 1977; Caton et al., 1984) and an increased blood flow to the uterine horn that contained placental tissue (Caton et al., 1984).

From the foregoing, it becomes apparent that under conditions of restricted oxygen supply, compensatory mechanisms exist that favor a better irrigation and oxygenation of the fetus. However, such compensation may be only partial or may occur in the best interest of the fetus.

Microsphere embolization of the utero-placental circulation in sheep resulted in fetal growth retardation (Clapp et al., 1981). This was associated with an abrupt cessation of normal increases in oxygen, glucose and lactate uptake observed in control animals and a reduction of fetal oxidative metabolic rate. These authors suggested that decreased fetal growth may represent a fetal compensatory mechanism in response to placental damage which, in this case, had survival value since it markedly reduced substrate requirements.

Indeed, depending on the nature of the insult, compensation which may occur at different levels (maternal, placental and/or fetal), appears to be governed ultimately by fetal survival. Experiments in which pregnant animals were maintained in a hot environment, sufficient to elevate deep body temperature of the dam for several hours daily, provide an example of this phenomenon. During gestation, a temperature gradient of 0.5 C from conceptus to maternal unit was maintained through heat exchange across the placenta (Abrams et al., 1969). As an internal organ, the uterus relied on blood flow to dissipate heat (Gwasdauskas et al., 1974). Maternal hyperthermia caused a decrease in uterine blood flow in pregnant sheep (Oakes et al., 1976) and in ovariectomized dairy cattle injected with estradiol (Roman-Ponce et al., 1978). Thus, as heat stress reduced the capability to dissipate heat at the uterine level, temperature of the uterine serosa increased (Gwasdauskas et al., 1974). In pregnant animals, this may result in loss of the temperature gradient between conceptus and maternal unit and fetal well-being may be compromised due to hyperthermia.

With this knowledge, it is not surprising that both birth weight and placental weight were reduced in sheep as a response to chronic heat stress (Alexander and Williams, 1971). Heat stress for the last two thirds of pregnancy produced fetuses of 1 kg in weight as compared to 4 kg in the control group. Likewise, weight of the cotyledons were reduced by 60 to 75% in heat stress sheep. In cows, calf birthweight (Collier et al., 1980) and placental weight (Head et al., 1981) were lower during the hot summer months, and heat stress during the last trimester of gestation reduced calf birth weight (Collier et al., 1982). Thus, when pregnant animals are heat stressed, the conceptus appears to reduce its metabolism and growth to prevent hyperthermia thereby assuring its survival. A similar type of response was observed when guinea pigs were exercised daily throughout pregnancy (Gilbert et al., 1979). In the process of heat dissipation during maternal exercise, blood flow to the uterus may be diverted to the periphery thereby forcing the conceptus to respond as in the heat stressed condition. Decreases in birth weight and placental weight following maternal exercise in guinea pigs were associated with a lower placental diffusing capacity to carbon monoxide (Gilbert et al., 1979) and a lower placental surface area (Smith et al., 1983).

Variation of fetal genotype has been another approach used to illustrate conceptus and maternal interactions during pregnancy. Early studies mostly emphasized the importance of the maternal unit in regulating fetal growth. Crossbreeding experiments between either two breeds of horses (Walton and Hammond, 1938) or cattle (Joubert and Hammond, 1958), that were markedly different in size, indicated that size of the offspring was related to that of the mother. In both studies, the constraint to intrauterine growth imposed by a smaller dam was more complete than the enhancement allowed by the larger dam. These observations led to the conclusion that maternal rather than fetal factors regulate fetal growth in utero. A maternal regulating system, involving hormones from the maternal pituitary, was postulated after factors such as uterine capacity, placental surface and maternal food supply were rejected (Walton and Hammond, 1938). On the other hand, it was recognized that maternal regulation in this type of experimental model may have obscured any genetic differences (Walton and Hammond, 1938) and that calf birth weight may be affected by fetal size (Joubert and Hammond, 1958).

Sire of fetus has been implicated as a factor controlling variability in calf birth weight (Joubert and Hammond, 1958; Boyd and Hafs, 1965), ease of calving, frequency of dystocia (Quesnel et al., 1979) and gestation length (Defries et al., 1959; Boyd and Hafs, 1965).

For example, heritability estimates from parental half-sib correlation was 0.40 which indicates that sire of fetus partially controls variability in gestation length. Furthermore, sire of fetus accounted for a portion of the variability in milk production (Skjervold and Fimland, 1975; Adkinson et al., 1977; Quesnel, 1978; Van Vleck and Johnson, 1980). In a review, Thatcher et al. (1980) described the various statistical methods used and reported that estimates of sire of fetus effects on milk yield ranged from essentially 0 to 11.8%. Therefore, the possibility exists that service sire influences the maternal unit via the conceptus she bears. Such conceptus effects on the maternal unit also are evident by the fact that gestation length was longer for male than female fetuses (Fitch et al., 1924).

In this section, evidence has been presented that both conceptus and maternal units express the capability to adjust physiologically to experimental manipulation of their relationship. Endocrine response associated with various alterations of conceptus and maternal interrelationships will be presented.

Endocrine Responses

Few studies have been conducted relating placental and hormonal changes to experimental manipulation of conceptus and maternal interrelationships. Maternal underfeeding caused reduction in placental and fetal weight in sheep, pigs and rats (Anderson, 1975; Alexander, 1978). Since administration of estrogens and progesterone to pigs and rats largely prevented the effect of undernutrition on the conceptus, despite maternal weight loss, it was proposed that these hormones may be involved in regulating growth of the conceptus (Anderson, 1975). Progesterone injections induced overgrowth in caruncles of sheep

(Alexander and Williams, 1966) and maternal concentrations of progesterone tended to be higher in ewes carrying twins or triplets than singlets (Emady et al., 1974). Caton et al. (1983) detected positive correlations between birth weight of lambs and amount of progesterone released (r = 0.69) or concentrations of estrogens in the uterine artery (r = 0.58). Following ligation of one uterine horn in sheep (Caton et al., 1984), the process of compensatory growth of the placentome was associated with an increase in uterine blood flow to the gravid horn and a higher concentration of progesterone in maternal peripheral blood. Perhaps, steroid hormones induce changes of the uterine vasculature. Nevertheless, dynamic endocrine changes are associated with functional alteration of conceptus and maternal interrelationships.

Attempts were made to relate changes in prepartum endocrine dynamics with conceptus function. Thatcher et al. (1980) illustrated the large variability in concentrations of estrone sulfate between cows during late pregnancy. Among 10 cows, concentrations of estrone sulfate in peripheral plasma varied between 5000 to 15000 pg/ml (3-fold) just prior to parturition and between 250 to 7000 pg/ml (28-fold) between 76 and 48 days prepartum. As estrone sulfate is presumably of conceptus origin (Hoffmann et al., 1979), these authors suggested that such variability may reflect differences in hormonal production by the conceptus which in turn may be associated with specific responses of maternal and fetal units. Consistent with this view, Collier et al. (1982) demonstrated that hormones of maternal and conceptus origin were altered by the environment in which cows were managed during the last third of pregnancy. They reported that heat stress during late gestation increased prepartum plasma progesterone concentrations, but reduced prepartum plasma concentrations of estrone sulfate. These endocrine changes may reflect either an increased secretion or reduced metabolism of progesterone produced by the corpus luteum and a lower production of estrogens by the conceptus.

Based upon the apparent effects of service sire on calving ease (Osinga, 1970; 1978) or postpartum milk production (Adkinson et al., 1977), it was hypothesized that the ability of the service sire to exert these effects on the maternal unit was mediated by hormones produced by the conceptus she bears. Increased calving difficulty was associated with lower rates of urinary excretion of estrogens (Chew et al., 1977; Osinga, 1978) and evidence was presented that the sire or breed of sire influenced the amount of estrogens produced by the conceptus (Osinga, 1978). Heritability of urinary excretion of estrone (estrone/creatinine ratio) and estradiol-17_α (estradiol-17_α/creatinine ratio) at 260 days of gestation were 0.28 and 0.38, respectively (Osinga, 1970). Therefore, variation in fetal genotype is associated, to some extent, with variation in excretion of estrogens during pregnancy.

Following observations made by Osinga (1970; 1978) and by Adkinson et al. (1977), Eley et al. (1981a; 1981b) reasoned that fetal genotype may influence hormonal balance of the maternal unit associated with physiological and productive traits of the dam. They evaluated periparturient endocrine changes of the maternal unit in two genetically diverse groups of Jersey cows selected for milk yield. Control cows sired and bred to bulls with assumed zero estimated breeding value and selected cows sired and bred to bulls of high predicted difference for milk yield were utilized to test this hypothesis. Since sire of cow and sire of fetus effects were confounded in this experiment, measurement in maternal circulation did not provide a clear estimate of sire of fetus effects alone. Nevertheless, selection for milk yield influenced both the conceptus and the maternal unit in that prepartum concentrations of progesterone were higher in selected cows but concentrations of estrone, LH and prolactin were lower than in control cows. Thus, genetically different conceptuses contribute to differences in hormonal milieu of the dam.

Evidence has been presented that in response to alterations (physical, environmental or genetic) of conceptus and maternal interrelationships, endocrine changes occur that may reflect adaptation of conceptus and/or maternal units to the environment via homeorhetic signals from the conceptus. These changes in endocrine dynamics of the maternal unit associated with variation in conceptus function, may reflect altered physiological processes of the maternal unit. In the following section, potential associations of variability in prepartum conceptus function to subsequent productive and reproductive responses of the dam will be presented.

Productive and Reproductive Traits of the Dam

Hormones secreted by both the conceptus and maternal units influence development of the mammary gland and lactogenesis (see reviews by Tucker, 1969; Erb, 1977; Delouis et al., 1980). In sheep, Rattray et al. (1974) showed that the extent of mammary gland growth was most pronounced during the period of pregnancy in which the fetus was growing rapidly and that number of fetuses carried by the dam was related positively with mammary gland development. In goats, total placentome weight was related to fetal (r = 0.73) and mammary gland (r = 0.71) weight (Hayden et al., 1979). By pooling data from two different studies, Thatcher et al. (1980) reported that growth of the bovine fetus and that of the mammary gland were essentially parallel during pregnancy. Collectively, these relationships may reflect a certain degree of control of mammary gland growth by the conceptus.

Kulhanek et al. (1974) provided evidence in sheep of a functional placental maturation during the period of maximal fetal growth. Placental permeability to urea measured at 90 days of pregnancy was only 20% of that measured at term. In addition, these authors demonstrated that at a given fetal age (140-150 days of gestation), placental permeability to urea per kilogram of fetal weight is less than normal when the placenta is small. Thus, it appears that placental function changes during late pregnancy and that a relationship exists between placental size and function. Furthermore, a close correlation has been demonstrated between fetal weight and placental weight in man (Thomson et al., 1969), sheep (Alexander, 1974; Kulhanek et al., 1974), pig (Wooton et al., 1977), rabbits (Bruce and Abdul-Karim, 1973) and cattle (Head et al., 1981). Influence of the conceptus on mammary gland development and milk production may, therefore, be related to placental or fetal weight in cattle.

Studies of large herd data sets indicated that calf birth weight was associated positively with milk production (Quesnel et al., 1979; Collier et al., 1980; Erb et al., 1980). For example, milk production increased 103 kg with birth weights ranging from 18.6 to 29.5 kg for Jersey and 157 kg for a range of 34.5 to 45.4 kg for Holstein cows (Collier et al., 1980).

Experiments in which conceptus function was altered provided further evidence that milk production may be influenced by the conceptus. Benson and Morris (1971) reported that pregnant rats subjected to heat stress during the last two thirds of gestation gave birth to pups of reduced weight and exhibited impaired lactation. In cattle, Collier et al. (1982) reasoned that heat stress also may affect placental function during pregnancy which may have negative effects on calf birth weight and milk production. As expected, heat stress caused fetal growth retardation and this was associated with alterations of prepartum endocrine dynamics of dam (progesterone, thyroxine, triiodothyronine) and conceptus (estrone sulfate). In addition to a nonsignificant decrease in milk production in heat stressed cows, there was a linear relationship between calf birth weight and subsequent milk yield of the dam (r = 0.54; Collier et al., 1982). It was suggested that the lower profile of estrone sulfate concentrations prepartum was indicative of a negative effect of heat stress on placental function which may have contributed to the lower calf birth weight and indirectly altered subsequent milk yield.

As demonstrated earlier, estrogens are products of conceptus origin and may possibly be related to the potential effect of the conceptus on mammary gland function. Relationships between concentrations of estrogens during gestation, calf birth weight and subsequent milk yield were reported. Mean plasma concentrations of estrone and estradiol-17_{β} were correlated positively with calf birth weight (r = 0.22 and r = 0.21, respectively) and estradiol-17_{β} was correlated with milk yield (r = 0.21; Erb et al., 1980). Terqui et al. (1975) reported that total estrogen concentrations (estrone sulfate + estrone + estradiol) at day 220 of gestation in Charolais cattle were correlated with birth weight (r = 0.84) and subsequent preweaning growth (r = 0.87). The latter response being associated presumably with milk production. On the other hand, Eley et al. (1981a) found negative correlations between milk production and immediate prepartum changes in estrone (r = -0.72) or estrone sulfate (r = -0.55).

Erb et al. (1980) detected a positive relationship between prolactin and estrone (r = 0.16) or estradiol-17_β (r = 0.22) among 167 cows. In addition, an increase in mean prepartum plasma prolactin concentrations from 50 to 195 ng/ml was associated with approximately a 15% increase in predicted milk production. Eley et al. (1981a) observed that the rise in prolactin, which was more closely synchronized with the last 3 days of gestation in cows selected for and bearing conceptuses selected for milk yield, was associated negatively with progesterone and positively with estrogens. These authors suggested that the dynamics of prolactin secretion may be involved in processes of mammogenesis and lactogenesis in dairy cows, and that conceptus production of estrogens or other conceptus products may be the link between the dam and her fetus.

Placental lactogen activity in placentomes increased with the number of fetuses in goats (Hayden et al., 1979). Evidence of mammogenic and lactogenic potencies of placental lactogen in sheep and goat was reviewed by Delouis et al. (1980). Higher concentrations of placental lactogen measured in dairy cows than beef cows (1103 versus

650 ng/ml) were interpreted as evidence that placental lactogen may stimulate milk production in dairy cows. However, other studies (Buttle and Forsyth, 1976; Kelly et al., 1976; Schellenberg and Friesen, 1981) indicated that virtually no placental lactogen was present in the maternal circulation. This casts doubt on the relationship established by Bolander et al. (1976). Nevertheless, since presence of placental lactogen in bovine placentome tissue has been confirmed in vitro (Kensinger, 1982; Arima and Bremel, 1982), the possibility remains that the conceptus may affect mammary gland growth directly or indirectly via this hormone.

There is some evidence that the prepartum endocrine dynamics of the dam may influence her reproductive performance postpartum. Adkinson et al. (1977) reported that sire of fetus accounted for 1.9 and 3.2% of the total variability for days open in Holstein and Jersey cows, respectively. Based on their hypothesis, this effect of sire of fetus would be transmitted to the dam via the conceptus she bears. Prepartum endocrine alterations associated with genotype of conceptus (Eley et al., 1981a; 1981b) and with prepartum environment during the last trimester of gestation (Collier et al., 1982; Lewis et al., 1984), had carryover effects on postpartum endocrine function and resumption of reproductive cycles in cattle. In both studies, genetic or environmental manipulations of conceptus and maternal interrelationships were associated with variation in postpartum profiles of PGFM concentrations, variation in postpartum ovarian activity (progesterone and ovarian structures), and in uterine involution. Thus, it becomes conceivable that effects of endocrine changes associated with conceptus and maternal interactions prepartum are carried over to affect functions of the maternal unit during the postpartum period.

Collectively, evidence presented in this section suggests that interaction between conceptus and maternal unit can be altered by various means (physical, environmental, genetical). These alterations are associated with variation in the dynamic of hormones of maternal and conceptus origin and by variation in production traits and reproductive function of the dam. In the next section, events related to processes of uterine involution and resumption of reproductive cycle during the postpartum period in cattle will be reviewed.

Postpartum Uterine Involution

Gross Morphological Changes

Immediately following delivery of the feto-placental unit, the large postpartum uterus undergoes dramatic morphological and histological changes that are essential to reestablishment of a uterine milieu conducive to initiating a new pregnancy. Information presented below was obtained largely from reviews by Kiracofe (1980), Moller (1970), Morrow et al. (1969) and Casida (1968).

Rectal palpation of the postpartum uterus has been the method used most commonly to estimate the time interval from parturition to complete uterine involution. The uterus was considered to be involuted when it had returned to its normal, nongravid position, and when the two uterine horns were similar in diameter, and were showing normal consistency and tonus (Casida, 1968), or when the two uterine horns have returned to nongravid size (Gier and Marion, 1968). Generally, one or more of these criteria have been used to study the process of uterine involution by rectal palpation of the uterus.

Intervals between palpations ranged from a few days to approximately two weeks.

Rasbech (1950) outlined a four-stage sequence (i.e., days 1 to 8, 8-10, 10-18, and 18-25) of uterine involution which can be followed by palpation. During the first 24 h postpartum, the vagina can be palpated but the cervix can barely be distinguished from the uterus until day 3. After this time, uterus regains sufficient tone and its surface feels hard and corrugated. The caruncles can be felt only through the uterine wall when it is relaxed. During the second stage (8-10 days), the entire uterus can be palpated and its surface feels smooth and soft with some fluctuations and caruncles can be detected. During the third stage (10-18 days), the uterus feels like a soft plastic body with caruncles and fluctuation becomes less pronounced. During the final stage (18-25 days), uterine tone increases and both uterine horns tend to become similar in size.

Time to complete uterine involution varies considerably in the literature. In 12 studies reviewed by Kiracofe (1980), estimates of intervals for complete uterine involution ranged from 28 to 56 days. Marion et al. (1968) conducted a large scale study of uterine involution with 385 dairy cows that were palpated twice weekly from 3 days after parturition until uterine involution was complete. Average interval from parturition to uterine involution, for all normal calving cows, was 39 days.

Factors Affecting Uterine Involution

Various physiological and environmental factors may influence uterine involution in cattle. Marion et al. (1968) reported that the average interval to uterine involution was longer for pluriparous

(40.6 days) than primiparous (34.0 days) cows. Similar observations also have been reported by Rasbech (1950), Buch et al. (1955), Quevedo et al. (1965) and Morrow et al. (1966). However, other studies indicated that age or number of previous pregnancies (Tennant et al., 1967) as well as breeds or types of cattle (Casida, 1968) had no effect on intervals to uterine involution.

A seasonal effect on uterine involution was noted (Marion et al., 1968). Generally, involution of the uterus was more rapid during the warm than cold seasons. However, if parturition occurred during extremely hot weather (38 C and above), then heat stress markedly increased the interval from parturition to complete involution of the uterus. The net effect of nutrition on uterine involution appeared to be minimal. High levels of energy or protein slightly hastened (Dunn et al., 1969) or did not have any effect (Kiracofe et al., 1969) on uterine involution.

Reports are conflicting regarding the effect of suckling on uterine involution. Wiltbank and Cook (1958) reported that suckling delayed uterine involution; Wagner and Hansel (1969) concluded that suckling did not hasten this process. However, in detailed morphological and histological studies of postpartum uteri collected at various intervals postpartum, Riesen et al. (1968) and Lauderdale et al. (1968) showed that suckling accelerated several processes involved in uterine involution.

Periparturient clinical problems such as dystocia, retained placenta, uterine infection, milk fever and ketosis tend to prolong uterine involution (Boyd, 1925; Rasbech, 1950; Buch et al., 1955; Gier and Marion, 1968; Tennant and Peddicord, 1968; Morrow et al., 1969; Fonseca et al., 1983). Gier and Marion (1968) estimated that abnormal clinical conditions may delay uterine involution by 5 to 8 days and speculated that these delays were due to a decrease in uterine motility immediately after parturition. They also suggested that these delays may be associated with the heavy leucocyte infiltration within the uterine wall.

Efforts to hasten the involutionary process of the postpartum uterus by utilizing therapeutic agents have had limited success. Administration of diethylstilbestrol (20 mg) within 9 h after parturition (Casida and Wisnicky, 1950) or daily physiological doses of estradiol-17_β (0.10 mg) during the involutionary period (Marion et al., 1968) did not alter the process of uterine involution. Ovariectomy was shown to shorten the interval to uterine involution in pluriparous cows, but treatment of ovariectomized cows with 0.05 mg of estradiol-17_β increased the interval to uterine involution (Marion et al., 1968). It was suggested that the reduced rate of uterine involution was due to a greater thickness of the uterine wall resulting from estrogen stimulation. On the other hand, Foote and Hunter (1964) reported that 10 mg of estradiol-17_β , injected intramuscularly on day 25 postpartum, shortened the postpartum interval to involution in pluriparous beef cows.

Reports on the influence of progestins on uterine involution also vary. Foote and Hunter (1964) indicated that daily injection of 50 mg progesterone given from 12 to 23 days after calving shortened the interval to complete uterine involution, but opposite results were presented by Fosgate et al. (1962) and Marion et al. (1968). Starting on the day of calving, administration of 100 mg of 17_α -hydroxyprogesterone on alternate days (Fosgate et al., 1962) or of 50 mg progesterone daily (Marion et al., 1968) prolonged the interval to complete involution of the uterus.

Hypophyseal stalk transection of beef heifers in mid-pregnancy did not appear to influence the subsequent postpartum uterine involution (Anderson et al., 1979). Britt et al. (1974) and Fernandes et al. (1978a) demonstrated that an injection of GnRH (Gonadotrophin Releasing Hormone) on day 14 postpartum accelerated the reduction in size of the uterus between 14 and 24 days postpartum. The increase in plasma progesterone concentrations noted after the GnRH injection led Fernandes et al. (1978a) to speculate that a change in uterine blood flow and water imbibition may have accelerated uterine involution.

Parturition is not an aseptic process and bacterial infection of the postpartum uterus is common (Elliot et al., 1968; Griffin et al., 1974; Studer and Morrow, 1978). Elliot et al. (1968) isolated thirty organisms including streptococcus, staphylococcus, micrococcus, corynebacterium, pseudomonas and escherichia from uteri collected between days 3 and 60 postpartum. However, the same study showed that recovery of bacteria decreased rapidly during the postpartum period. Over 93% of the uteri collected within 15 days after parturition were infected, and this percentage decreased to 78, 50 and 9% in uteri collected between 16 and 30, 31 and 45, and 46 and 60 days, respectively. Kiracofe et al. (1973) showed no effect of intrauterine innoculation with lactobacillus acidophilus on uterine involution of postpartum cows. Treatment of the uterus with enzymes or antibiotics frequently prolonged the time to complete involution (Seguin et al., 1974; Oxender and Seguin, 1976).

Histological Changes

Studies were conducted in which uteri were recovered at various intervals postpartum to characterize the variety of anatomical and histological changes associated with the involuting uterus. Gier and Marion (1968) described these changes as three overlapping processes: 1) reduction in size, 2) loss of tissue and 3) repair of tissue. The following discussion describes these processes through the major changes occurring in the intercaruncular and caruncular endometrium and in the myometrium. The reader is referred to Johanns et al. (1967), Gier and Marion (1968) and Wagner and Hansel (1969) for illustrations of postpartum histological changes and to Riesen et al. (1968) and Lauderdale et al. (1968) for quantitative analyses of these changes.

Weight of the whole uterus and length of the previously gravid uterine horn decreased in a logarithmic manner during the postpartum period (Gier and Marion, 1968). The decrease in weight and diameter of the uterine horns was more rapid during the initial phase (days 1 to 20) of uterine involution, and rate of change was greater for the previously gravid than nongravid uterine horn (Riesen et al., 1968; Lauderdale et al., 1968). By day 5 postpartum, the previously gravid uterine horn contracted to half of its original diameter and by day 15 to half of its original length (Gier and Marion, 1968). Morrow et al. (1969) suggested that the early reduction in size of the uterus resulted from vasoconstriction and peristaltic contractions that persist for several days (Jordan, 1952; Venable and McDonald, 1958). According to Gier and Marion (1968), loss of tissue fluid appeared to be an important factor involved in reduction of uterine size. Uterine lochial fluid was composed of both normal and degenerated cells from the tunica mucosa and caruncles and from bleeding occurring during shedding of the fetal membranes (Moller, 1970). Volume of uterine fluid or lochia recovered was maximum (800 to 2000 ml) during the first 8 days postpartum and then decreased progressively (75 to 750 ml) and was absent by day 24 postpartum (Gier and Marion, 1968).

During the period of rapid decrease in uterine weight and size, intrauterine tissues undergo marked structural changes. On the day after calving, the intercaruncular endometrium became highly edematous. This condition regressed slowly during the next 4 to 5 days. By day 8, endometrial edema had receded and the endometrium was folded leaving the caruncles in a general "stalked" appearance (Gier and Marion, 1968). Riesen et al. (1968) reported that the earliest portion of the postpartum interval (approximately the first 20 days) was marked by destruction of endometrial tissue that was accompanied by presence of large numbers of leucocytes and reduction in endometrial vascular bed. Wagner and Hansel (1969) indicated that the intercaruncular endometrial epithelium (day 14 postpartum) was highly proliferative in appearance, had lost its normal columnar arrangement, and was several cells thick.

The myometrium also undergoes its most dramatic changes during the first 20 to 30 days postpartum. The muscle layers (circular and longitudinal) decreased in mass both by reduction in cell size and number (Riesen et al., 1968; Lauderdale et al., 1968). Archbald et al. (1972) described the histological changes in the myometrium as consisting of a degeneration of sarcoplasm, vacuolation of the muscle cells, and atrophy of the nucleus. These processes were initiated by day 3 postpartum; the muscle fibers appeared normal and the entire myometrium was greatly reduced in size by day 31. They also indicated that there was no necrosis of muscle fibers during regression of the myometrium. The observation (Riesen et al., 1968; Lauderdale et al., 1968) that suckling enhanced the rate of involution of the three major uterine tissue layers (endometrium, and both circular and longitudinal myometrial muscle fibers) is noteworthy.

In cattle, expulsion of the fetal membranes usually occurs within 12 h after parturition, and the maternal part of the placentome (caruncle) eventually is lost during the postpartum period. On the day after calving, caruncles, which are more numerous in the previously gravid uterine horn, averaged 70 mm in length, 35 mm in width and 25 mm in height. They consisted primarily of a crumpled mass of septum and blood vessels capping an endometrial base (Gier and Marion, 1968). These authors described the gradual disappearance of the caruncular endometrium as follows: by day 3 postpartum, caruncles in the previously gravid uterine horn did not show appreciable changes in either size or tissue organization from the preparturient condition; only by day 5, necrosis and complete septal disorganization was associated with vasoconstriction and leucocytes infiltration; by day 12, most of the caruncular septum had sloughed, leaving a raw surface with protruding remnants of blood vessels; by day 15, sloughing was completed down to the stratum compactum; by day 19, caruncles appeared as rough knobs (15 to 20 mm in diameter) each containing a regressing mass of blood vessels. This regressive process of caruncular mass was observed until 40 to 60 days postpartum.

Histological studies further substantiated the previous gross observations and also indicated that the necrotic stage is not attained before day 5 postpartum after which time degeneration of the caruncular mass occurred rapidly. Archbald et al. (1972) described the sequence of events involved in regression of the caruncular mass as being initiated by degenerative vascular changes which are followed by peripheral ischemia, necrosis and sloughing. Progressive degenerative vascular changes were observed from days 1 to 19 postpartum. These changes consisted of hydropic degeneration of the cytoplasm and pycnosis of the smooth muscle cells of the tunica media and fibrinoid necrosis of the tunica media. Vascular changes were confined to the tunica media and luminal patency was a constant finding. Although vascular changes were initiated on the day after parturition, necrosis was not observed until day 5 postpartum. An epithelium was still covering the caruncular surface on days 1, 3 and 6, and only after the necrotic process had been initiated was the superficial layer of the caruncle with its epithelium sloughed (Archbald et al., 1972). Wagner and Hansel (1969) indicated that the sloughing of the caruncular endometrium appears to begin at levels just basal to the crypt.

Through radiographic examinations of involuting uteri, Hutchinson (1962) and Johanns et al. (1967) illustrated that vasoconstriction of the caruncular blood vessels leads to progressive regression of the caruncular endometrium. On the day after calving, Hutchinson (1962) observed the same level of tortuosity of blood vessels than was seen previously during late gestation. Vasoconstriction of the caruncular blood vessels, which occurred within 12 h after parturition, was not seen uniformly among the different caruncles (Hutchinson, 1962;

Johanns et al., 1967). Some caruncles showed an almost complete lack of a blood supply while others maintained the major vascular appearance observed during pregnancy (Hutchinson, 1962). On day 7 postpartum, size of the caruncle was reduced appreciably, and caruncular arteries were highly constricted or even in a state of degeneration (Johanns et al., 1967).

Moller (1970) identified two pathways by which waste products of uterine involution were eliminated. The regressing cells of the tunica serosa and subserosa, stratum vasculare and myometrium were removed by resorption via the vascular system. In contrast most of the epithelial cells of the uterine mucosa, from the intercaruncular and caruncular endometrium, were shed by desquamation into the uterine lumen and became part of the lochial fluid.

Tissue loss and repair occur simultaneously in the early postpartum period, but gradually the process of tissue repair becomes predominant and the uterine mucosa is restored. Archbald et al. (1972) indicated that prior to day 15 postpartum, both degeneration and regeneration of epithelial cells occurred in the intercaruncular endometrium; after this time, no degenerative epithelial cells were observed. The regenerated cells seemed to originate from the basal area of the endometrial epithelium and at no time during uterine involution was the intercaruncular area devoid of an epithelial layer. At some variance with these observations, Gier and Marion (1968) reported an almost immediate regeneration of the intercaruncular epithelium, in areas not seriously damaged during parturition, through replacement of endometrial epithelium by epithelium of the uterine endometrial glands. They also noted that the intercaruncular endometrium was partially or completely destroyed if bacterial infections occurred during the period of tissue loss. However, by day 5 postpartum, the entire intercaruncular area was covered by a new epithelium (Gier and Marion, 1968). The pattern of simultaneous degeneration and regeneration of epithelial cells of the uterine endometrial glands was similar to that described in the intercaruncular area (Archbald et al., 1972). The latter attributed this similarity to the common embryological origin of these two types of epithelium.

Regeneration of epithelium over regressing caruncles occurred from the margin (Wagner and Hansel, 1969). During the process of regeneration, cell height of the new epithelium gradually increased to the normal columnar arrangement. Gier and Marion (1968) noted, that by day 13, flat or cuboidal cells partially covered the caruncular area. Although Archbald et al. (1972) estimated that this process was completed by day 19 postpartum, Gier and Marion (1968) found that (even under the most favorable conditions), the caruncular surface was not covered by epithelium before day 25. Wagner and Hansel (1969) observed that regeneration of the caruncular surface was beginning by day 12 to 14 postpartum, and the caruncular surface was covered with an epithelial layer on day 30 postpartum. At the end of the repair process (day 40 to 60), caruncles regressed to smooth, oblong, epithelial-covered, avascular knobs (4 to 8 mm in diameter and 4 to 6 mm high) and appeared as rows of white discs in a pink and glandular endometrium (Gier and Marion, 1968). These authors also indicated that, at this time, regressed caruncles differed from virgin caruncles by their greater diameter, black pigmentation, more massive vascular bed at the base, and irregular margins.

Anatomical and histological involutionary changes which continue to occur until day 50 to 60 postpartum were related to a slight reduction in uterine size (Gier and Marion, 1968). Although the final reduction in size of the uterus may not be detectable by rectal palpation (Marion et al., 1968), this latter method provides a good estimation of the reduction in uterine size during the first 40 days postpartum.

Fertility

The degree of involution required to support another pregnancy has not been established. Wagner and Hansel (1969) indicated that the uterine mucosal epithelium was restored by day 30 and suggested that cows could be mated well before 60 days postpartum. Indeed cows conceived before uterine involution was completed (Buch et al., 1955; Perkins and Kidder, 1963; Moller, 1970; Odde and Kiracofe, 1978). However, if cows were bred prior to day 30 postpartum, conception rates were rather low (Perkins and Kidder, 1963; Casida, 1968; Kiracofe, 1980). On the other hand, Perkins and Kidder (1963) reported that after 40 days postpartum, conception rate was unaffected by the involutionary state of the bovine uterus.

Casida (1968) suggested that low fertility in the early postpartum period was due to lack of fertilization, since embryonic mortality did not differ between cows bred early (4 to 6 weeks postpartum) or at later stages (greater than 8 weeks postpartum). He proposed that in the early postpartum period, uterine conditions such as large physical size of the uterus, large endometrial surface, degenerating caruncles, tissue repair and high count of infiltrating leucocytes may be related to a lower fertility.

Differences in fertilization between the previously gravid and nongravid uterine horn were noticed. Saiduddin et al (1968) indicated that interval to breeding was the primary fertility factor if ovulation occurred on the ovary contralateral to the previously gravid uterine horn. However, the degree of involution of the previously gravid uterine horn was an important fertility factor if ovulation was to occur on the ovary ipsilateral to the previously gravid uterine horn. Therefore, a physiological state that favors fertilization is restored sooner in the previously nongravid than gravid uterine horn.

Physiologic and Hormonal Changes

Few studies have been conducted to understand the basic mechanisms involved in the processes of uterine involution. Information of this sort is available only for laboratory animals. A major feature of uterine involution in the rat is the reorganization of the connective tissue framework. Four days following parturition, more than 85% of uterine collagen was resorbed (Harkness and Moralee, 1956). Wray (1982) showed that loss of distension from the uterine wall is a primary factor involved in involution of the rat uterus. Distension of the uterus, by an inert material (Silgel), prevented involution and collagen loss. After removal of the distending material at 4 days postpartum, involution and collagen loss occurred. In addition, this author suggested that ovarian steroids may influence the rates at which collagen loss and involution proceed. However, loss of uterine distension was the primary stimulus to initiate these processes.

To the best of the author's knowledge, there are only three reports in cattle in which uterine involution have been studied in relation to the changes in the postpartum hormonal environment. Eley et al. (1981b) showed that the decrease in size of the previously gravid uterine horn was positively related to the decrease in peripheral plasma PGFM concentrations. Lindell et al. (1982) reported that, among clinically normal postpartum cows, those exhibiting a longer period of elevated peripheral plasma PGFM concentrations had a shorter interval to completed uterine involution. These authors suggested that $PGF_{2\alpha}$ may hasten involution by increasing smooth muscle contractions and uterine tone and long involution times may be due to an insufficient synthesis of $PGF_{2\alpha}$. In the same study, cows with variable periods of uterine discharge during the first 30 days postpartum had a longer period of elevated plasma PGFM concentrations and a longer interval for completion of uterine involution. In a different study, Lewis et al. (1984) reported that cows with a higher magnitude of peripheral plasma PGFM concentrations early postpartum had a shorter interval of uterine involution. This shorter interval was assessed as an earlier return of the uterus to its nongravid position. The postpartum profile of PGFM concentrations was higher in cows heat stressed during the last trimester of gestation. Authors suggested that this may reflect adaptations of conceptus and/or maternal units to their prepartum environment. Such responses introduce the possibility that prepartum conceptus and maternal interactions may affect postpartum uterine involution via a change in the profile of postpartum PGFM.

Resumption of Ovarian Activity

As compared to any other reproductive status during adult life of a cow, follicular development observed on the surface of ovaries at parturition was minimal (Casida, 1968). However, ovarian competence for follicular activity is resumed early postpartum. In general, first ovulation usually occurs within 3 weeks postpartum for non-suckled dairy cows. In the following discussion, local utero-ovarian and central hypothalamic-pituitary factors involved in resumption of ovarian activity are presented.

Ovarian Morphology

Average diameter of the largest follicle decreased from 12 mm in the second month of pregnancy to approximately 9 mm and 4 mm at the fifth and ninth month, respectively (Casida et al., 1943). Several studies (Foote and Peterson, 1968; Marion and Gier, 1968; Saiduddin et al., 1968; Wagner and Hansel, 1969; Stevenson et al., 1983) indicated that large follicles (10-12 mm in diameter) were visible on the ovarian surface within the first 2 weeks postpartum. Kesler et al. (1978) and Webb et al. (1980) were able to detect, by rectal palpation, ovarian follicles 4-5 mm in diameter as early as 4-5 days postpartum. Total number of follicles and total follicular fluid weight of the largest follicle increased appreciably from days 1 to 10 postpartum (Saiduddin et al., 1968).

Folliculogenesis is a continuous process during the lifetime of a female, but the rate at which it proceeds appears to be influenced by the physiological state of the animal. For example, histological studies of serially sliced ovaries (Cahill and Mauleon, 1980; Dufour and Guilbault, 1984) indicated that the end of the breeding season in sheep, ovarian status was characterized by a build-up of preantral follicles and a depletion of the antral follicular population.

Recently, a histological study of the dynamics of follicular growth illustrated a high degree of follicular activity in the population of small antral follicles during the early postpartum period in cattle (Dufour and Roy, 1983). Between days 15 and 35, there was a complete depletion of small antral follicles (0.16 to 0.28 mm diameter) accompanied by a gradual replenishment of larger follicles (0.19 to 0.67 and 0.68 to 1.57 mm classes). Estimates of follicular growth, from antrum formation to preovulatory size, were about 45 days in sheep (Turnbull et al., 1977) and 41 days in cows (Lussier et al., 1983). First ovulation postpartum, usually occurred within the first 3 weeks in the majority of non-suckled dairy cows (Marion et al., 1968; Stevenson and Britt, 1980). Thus, first ovulation postpartum involved follicles that were recruited from a pool of growing follicles early postpartum (Dufour and Roy, 1983). The anovulatory postpartum period in cattle was characterized by increased follicular growth and a coincident increase in the ability of the largest follicle to produce estradiol (Spicer et al., 1983). This suggests that ovarian follicular development may be altered during the postpartum period. Factors controlling ovarian follicular development and ovulation will be presented.

Hypothalamo-pituitary Axis

Follicular growth during the early postpartum period apparently resulted from surges of FSH secretion, at a time when LH (in pituitary gland and plasma) was relatively low. During late pregnancy, evidence in ewes (Chamley et al., 1974) and cows (Schallenberger et al., 1978;

Azzazi et al., 1983) suggested that LH synthesis and secretion at the hypothalamo-pituitary axis is suppressed by high levels of estrogens and progesterone. In contrast, FSH synthesis was not altered, but its secretion was suppressed by high endogenous steroid levels. Following parturition, pituitary content of FSH and LH were inversely related. While pituitary LH content increased between days 1 to 20 postpartum, pituitary FSH content decreased (Saiduddin et al., 1968) and the latter was reflected by an early postpartum (day 5) increase in plasma FSH concentrations (Dobson, 1978; Schams et al., 1978). There is general agreement (Schams et al., 1978; Lamming et al., 1981; Schallenberger et al., 1982), that once the increase in FSH has occurred after parturition, plasma FSH concentrations are not a limiting factor influencing onset of ovarian cycles postpartum.

In cyclic cows, increases in basal LH concentrations from luteolysis until the preovulatory surge (Chenault et al., 1975; Schams et al., 1977) reflected an increase in pulsatile LH secretion, which reached a threshold frequency of about 1 pulse/h just before ovulation (Rahe et al., 1980). It appears that the pulsatile profile of LH release was due to pulsatile stimulation of the anterior pituitary by GnRH (Knobil, 1980). In early postpartum cows, low LH concentrations (Echternkamp and Hansel, 1973; Kesler et al., 1977; Fernandes et al., 1978b; Schams et al., 1978; Goodale et al., 1978; Webb et al., 1980; Eley et al., 1981a) appeared to reflect malfunction of the hypothalamo-pituitary axis relative to LH release. In response to a challenge with GnRH injection, ability of the pituitary to release LH was not regained before day 10 postpartum (Kesler et al., 1977; Webb et al., 1977; Fernandes et al., 1978b; Schallenberger et al., 1978; Foster et al., 1980).

The increase in basal concentrations of LH from days 2 or 3 postpartum (Echternkamp and Hansel, 1973; Kesler et al., 1977; Schams et al., 1978; Goodale et al., 1978; Webb et al., 1980; Eley et al., 1981a) was due to an increase in magnitude and frequency of LH release (Goodale et al., 1978; Carruthers and Hafs, 1980; Peters et al., 1981; Schallenberger and Peterson, 1982). Lamming et al. (1981) stated that an episodic pattern of LH release at the proper magnitude and frequency has to be resumed before cyclicity recommenced. The frequency of LH release for the acyclic anestrus dairy cow revealed a steady increase from days 4 to 32 postpartum (Schallenberger and Peterson, 1982). Schallenberger et al. (1982) estimated that a pattern of LH release that can initiate cyclic function is resumed within 10 to 32 days postpartum in milked dairy cows. In a preliminary experiment, Schallenberger et al. (1982) also indicated that priming of the pituitary gland with intermittent hourly infusions of 500 ng GnRH, from parturition onward, induces pulsatile release of LH after 5 days postpartum. The importance of a priming period with GnRH to induce pituitary and ovarian function has been demonstrated in monkeys (Knobil, 1980). Priming with GnRH pulses increased sensitivity of bovine pituitary cells to GnRH in vitro (Padmanabhan and Convey, 1981).

Evidence strongly suggests that suckling has a depressive effect at the hypothalamo-pituitary axis which causes delay in onset of estrus and ovulation in beef and dairy cows (Short et al., 1972). Serum LH concentrations during the early postpartum period were higher in nonsuckled than suckled cows (Short et al., 1972; Randel et al., 1976). After weaning, serum LH concentrations increased (Smith et al., 1977) and reflected an increase in frequency of pulsatile LH release (Forrest et al., 1979). Suckling reduced both frequency and amplitude of the episodic release of LH in dairy (Carruthers and Hafs, 1980) and beef cows (Walters et al., 1982a; 1982b; 1982c). Estrus and ovulation occurred sooner in suckled cows that received intermittent small-dose injections of GnRH that mimicked the pattern of pulsatile LH release observed in weaned cows (Walters et al., 1982). From these results, it was hypothesized that suckling prolongs the postpartum interval by reducing the frequency of pulsatile GnRH release from the hypothalamus. Ability of pituitary cells to secrete LH in response to GnRH was reduced by cortisol (Wagner and Li, 1982). These authors suggested that the increased secretion of cortisol by adrenal glands in suckled cows may be involved in the mechanism by which suckling causes a delay in resumption of ovarian function in cattle. This has yet to be conclusively documented. Other factors such as season, nutrition, and stress can also influence the onset of estrus and ovulation (Lamming et al., 1981).

Hypothalamo-pituitary-ovarian Axis

By developing the ability to produce estrogens, growing follicles exert a positive feedback on secretion of gonadotropin which in turn further increases follicular estrogen production and follicular responsiveness to gonadotropins (Richards and Midgley, 1976). Of all the follicles that are in a growth phase, only a few ovulate while a large majority undergo atresia. Large fluctuations in plasma estradiol concentrations were observed prior to the first ovulation postpartum.

This may reflect simultaneous follicular growth and atresia during this early period (Echternkamp and Hansel, 1973; Fernandes et al., 1978b; Stevenson and Britt, 1979).

The role of ovarian hormones, primarily estrogens, in the process of inducing the gonadotropin surge and ovulation has been established. Treatment of ovariectomized cows with estradiol, at concentrations that simulated those found during proestrus, induced preovulatory-like surges of LH and FSH (Kesner et al., 1982). After removal of the suckling stimulus in postpartum beef cows, Walters et al., 1982a; 1982b) provided evidence that the increase in pulsatile LH release causes an increase in LH and FSH receptors of the largest ovarian follicle and an increase in estrogen secretion. In a preliminary experiment, priming of the pituitary with GnRH pulses from parturition onward permitted an injection of estradiol-benzoate on day 5 postpartum to elicit a LH surge which was followed by ovulation (Schallenberger et al., 1982). Collectively, these results indicate that reestablishment of a functional hypothalamo-pituitary-ovarian axis is necessary for a postpartum ovulation to occur.

In both beef and dairy cows, corpora lutea resulting from the first ovulation postpartum have a smaller size, a shorter life span, and were associated with lower plasma concentrations of progesterone than those resulting from ovulation in a normal cyclic cow (Menge et al., 1962; Morrow et al., 1969; Kesler et al., 1978; Stevenson and Britt, 1979; Ward et al., 1979). Kesler et al. (1981) reported that the in vitro progesterone response to LH by bovine corpora lutea harvested 7 days after a GnRH challenge (between days 27 and 29 postpartum) was lower than that observed from corpora lutea harvested on day 7 of the estrous cycle. It was suggested that short luteal phases are not related to a lack of gonadotropin support after first ovulation but rather a failure of corpora lutea to recognize LH.

Abnormal ovarian follicular maturation before ovulation was proposed to explain occurrence of short luteal phases (Kesler et al., 1978; 1981) or of short increase in plasma progesterone concentrations (Lamming et al., 1981). Shirar and Martinet (1982) stated that asynchrony between ovarian and pituitary hormones may alter follicular growth and maturation during the early postpartum period. This would appear likely in light of the fact that first ovulation postpartum involved follicles that were recruited from a pool of growing follicles early postpartum (Dufour and Roy, 1983).

Utero-ovarian Axis

Local utero-ovarian factors also appear to influence follicular development and ovulation during the postpartum period. A high percentage of ovulations in the first 20 days postpartum occurred on the ovary contralateral to the previously gravid uterine horn (Saiduddin et al., 1967; Foote and Peterson, 1968; Kiracofe et al., 1969). Marion and Gier (1968) reported that over 90% of first ovulations that occurred before day 15 and 60% of those between days 15 and 20 postpartum were found on ovaries contralateral to the previously gravid uterine horn. After the previously gravid uterine horn diameter has decreased to less than 60 mm diameter, these authors reported that the local influence of this uterine horn was no longer evident on frequency of ovulation. Wagner and Hansel (1969) were unable to confirm these observations.

Marion and Gier (1968) suggested that cytological endometrial disturbances in the previously gravid uterine horn during early uterine involution may inhibit ovulation and corpus luteum development. Foote and Peterson (1969) proposed that the previously gravid uterine horn may inhibit or "fail to stimulate" the adjacent ovary, and this influence diminished as both horns reached similar size. On the other hand, intraovarian factors have been proposed to explain the high incidence of ovulation on ovaries contralateral to the previously gravid uterine horn. Presence of the regressing corpus luteum of pregnancy (Foote and Peterson, 1968) or the high intraovarian levels of progesterone secreted by the corpus luteum during pregnancy (Shirar and Martinet, 1982) were suggested as factors involved in causing an unilateral block of follicular development and maturation. Indeed, Casida and Venzke (1936) reported that postpartum development of follicles to half mature size (12 mm diameter) was approximately 8 days longer in the ovary which contained the corpus luteum of pregnancy than in the opposite ovary.

Lamond and Drost (1974) suggested that an abnormal anatomical relationship between uterine and ovarian vasculature, adjacent to the previously gravid uterine horn may preclude proper ovarian follicular development during the early postpartum period. Indeed, the uterine branch of the ovarian artery (UBOA) which is an anastamosis between uterine and ovarian blood supplies (Mapletoft et al., 1976), allows flow of blood in both directions depending on uterine vascular resistance (Lamond and Drost, 1974; Ford and Chenault, 1981). When uterine vascular resistance is low (estrus or gestation: Ford and Chenault, 1981; Ferrell and Ford, 1980), flow of blood in the UBOA would be directed toward the uterus. In contrast, when uterine vascular resistance is high (luteal phase or postpartum: Ford and Chenault, 1981; Ford et al., 1982), blood in the UBOA would flow in the ovarian direction. Thus, during the postpartum period, blood supply to the ovary via the enlarged UBOA (Lamond and Drost, 1974) may exceed its need and alter ovarian function.

Cows that bore genetically different conceptuses (Eley et al., 1981a; 1981b) or that were managed in a shade or no shade environments prepartum (Collier et al., 1982; Lewis et al., 1984) exhibited different ovarian activities during the postpartum period. Notably, Lewis et al. (1984) reported that prepartum heat stress attenuated the negative effect of the previously gravid uterine horn on postpartum activity of ovaries ipsilateral to this horn. Interestingly, the latter was associated with prepartum variations in estrogen and progesterone profiles (Collier et al., 1982) and in a higher magnitude of postpartum PGFM concentrations (Lewis et al., 1984) that may reflect conceptus-maternal interactions.

Prostaglandins facilitated gonadotropin secretion by stimulating GnRH release from the hypothalamus (Spies and Norman, 1973; Harms et al., 1974; Chobsieng et al., 1975; Drouin et al., 1976; Labrie et al., 1976; Roberts et al., 1976). Venous-arterial differences in concentrations of $PGF_{2\alpha}$ were measured across the brain of the anestrous sheep (Roberts et al., 1976). Results indicated that the brain, possibly the hypothalamus, was a significant source of $PGF_{2\alpha}$ and that pulsatile release of $PGF_{2\alpha}$ may modulate the release of LH. Indomethacin, an inhibitor of prostaglandin synthesis, inhibited the estradiol-induced release of LH. Furthermore, infusion of $PGF_{2\alpha}$ in the carotid artery (6.0 ug/h) induced a sharp increase in concentrations of LH in jugular venous plasma. It was indicated also in this study that a large pulse of $PGF_{2\alpha}$ preceded the LH surge. Thus, large concentrations of F series prostaglandins measured in plasma of early postpartum cows may influence the release of LH during this period.

The role of prostaglandins in ovarian recrudescence is unclear, but they also have been implicated as a local factor involved in folliculogenesis (Armstrong, 1981; Tam and Roy, 1982). Synthesis of $PGF_{2\alpha}$ was suppressed by hysterectomy or injections of indomethacin in guinea pigs (Tam and Roy, 1982). Such treatment delayed ovulation and this was associated with an absence of antral follicles greater than 0.8 mm in diameter in a majority of animals (26/28) near the time of ovulation. Replacement therapy with $PGF_{2\alpha}$ stimulated the development of follicles from 0.8 to 1.0 mm in diameter in preparation for the final rapid preovulatory growth. It was suggested that $PGF_{2\alpha}$ acts on developing follicles for a given period before they are capable of ovulation and that $PGF_{2\alpha}$ is one of the factors required to stimulate growth of follicles at the end of the estrous cycle.

A high incidence of silent heat was reported at first ovulation (Morrow et al., 1966; Saiduddin et al., 1967; 1968; Marion and Gier, 1968; Kiracofe, 1980). Casida (1968) reported that the degree of estrous behavior increased as the postpartum interval increased. Thatcher and Wilcox (1973) reported that the fertility of dairy cattle inseminated at 60 days after parturition increased as the frequency of prior detected estrous periods increased during the postpartum period. Association of estrous frequency and fertility was due to occurrence of estrous periods during the first 30 days after calving. Consequently, early restoration of ovarian function appears to improve subsequent reproductive efficiency.

Present review illustrated some of the elements that may potentially affect postpartum reproduction in cattle and that are related to prepartum conceptus and maternal interactions. It was in this spirit that this research was undertaken.

CHAPTER III
SOURCE OF F SERIES PROSTAGLANDINS DURING THE EARLY POSTPARTUM PERIOD IN CATTLE

Introduction

Increases in prostaglandin concentrations during the periparturient period in human (Green et al., 1974), laboratory animals (Labhsetwar and Watson, 1974) and domestic animals, such as sheep (Liggins et al., 1973) and goats (Currie, 1975), have been related to the sequence of hormonal changes that initiates normal labor and parturition (Nathanielsz, 1978; Thorburn and Challis, 1979; Challis and Mitchell, 1981). In the cow, as reflected by peripheral concentrations of 15-keto-13,14-dihydro-$PGF_{2\alpha}$ (PGFM), similar increases in prostaglandin extended into the postpartum period (Edqvist et al., 1978; Eley et al., 1981b; Lindell et al., 1982). However, the role of prostaglandins during the postpartum period is poorly understood, and their site of production is unknown since only peripheral measurements of PGFM were obtained.

Prostaglandins are synthetized by virtually every tissue in the body (Christ and Van Dorp, 1972). If not metabolized directly at the site of synthesis, native prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is metabolized almost entirely into PGFM upon a single passage through the lungs (Ferreira and Vane, 1967; Davis et al., 1980; Maule Walker and Peaker, 1981). Therefore, the pool of peripheral plasma PGFM might be derived from local tissue metabolism of $PGF_{2\alpha}$ at the site of production or from metabolism after $PGF_{2\alpha}$ has been released into the general circulation. Alternatively, peripheral PGFM might originate from a more specific organ during the periparturient period in the cow. Maule Walker and Peaker (1981) indicated that the mammary gland of the goat was a site of production and metabolism of $PGF_{2\alpha}$ during the periparturient period, and a positive venous arterial difference in PGFM concentrations was maintained across the mammary gland for at least 10 days postpartum. In vivo and in vitro experiments also indicated that the uterus is a very active site of $PGF_{2\alpha}$ production during late pregnancy and after labor in sheep and goat (Liggins and Grieves, 1971; Keirse et al., 1976; Mitchell et al., 1976; Keirse et al., 1977; Mitchell and Flint, 1978b; Mitchell et al., 1978; Mitchell et al., 1980; Evans et al., 1982). During the periparturient period of cattle, temporal relationships exist which suggest an association between demise of the CL and increased prostaglandin concentrations (Fairclough et al., 1975; Edqvist et al., 1978; Eley et al., 1981b). Furthermore, increases in PGFM are closely related to calf and placental delivery, and decreases in uterine horn diameter was correlated positively with decrease in postpartum PGFM concentrations (Eley et al., 1981b; Lindell et al., 1982). Collectively, these observations suggest a uterine source of $PGF_{2\alpha}$ and/or PGFM during the postpartum period.

Objective of the present experiments was to determine contribution of the uterus to the postpartum pool of PGFM. This was accomplished by performing complete hysterectomy of a prolapsed uterus during the early postpartum period and measuring the capability of intrauterine tissues to synthesize and release prostaglandins in vitro.

Materials and Methods

Experiment 1

Animals and surgery

On the day of parturition (day 0), 15 cows were assigned to either a control group (untreated; n=4), a hysterectomized group (n=8), or a sham-operated group (n=3). Surgery was performed on cows assigned to the hysterectomized and sham-operated groups within 8 h after parturition. At this time, fetal membranes had been expelled and the cervix was still dilated. Animals were restrained in a stanchion and an indwelling polyvinyl catheter (0.86 mm id x 1.52 mm od; V-6, Bolab Inc., Derry, NH) inserted (16-18 cm) into the jugular vein. The vulva was thoroughly washed with soap and water, and the cervix examined manually for degree of dilation. To relax the uterus, a slow I.V. infusion of epinephrine (1:25,000; 250 ml) in sterile physiological saline was given to each cow. Relaxation of the uterus occurred within 1 to 2 min after onset of epinephrine infusion and frequently was complete by the time 100-150 ml of solution was administered. Several large caruncles were grasped in the distal portion of the previously gravid horn, and the flaccid uterus inverted and retracted into the vagina. Presence of the uterine mass in the vagina induced straining by the cow and permitted the operator to reach other caruncles closer to the tip of the previously gravid uterine horn. With steady, firm but gentle traction, the entire horn was invaginated, and subsequently the entire uterus prolapsed. As soon as the prolapse was complete, epinephrine infusion was terminated and epidural anesthesia (5 ml, 2% lidocaine hydrochloride; Med Tech Inc., Elwood, KS) was given to suspend further straining.

Hysterectomy of a prolapsed uterus was done by the method of Sloss and Dufty (1980). A 25 cm incision, starting at the level of the posterior cervix, was made through the dorsal wall of the prolapsed uterus. The lumen of the prolapsed uterus was inspected for presence of ovaries and/or abdominal viscera, and main uterine arteries doubled ligated with catgut. Total uterine blood supply was occluded by a final ligature of strong rubber tubing placed around the base of the prolapse at the junction of the uterus and posterior cervix. Rubber tubing was transfixed to the cervix in four locations and approximately 80% of the cervix remained intact. The prolapsed uterus remained suspended 12 h to allow blood clots to form in the ligated vessels, and then amputation of the uterus was performed.

In sham-operated cows, uteri also were prolapsed; however, no incision or ligation was made and the entire uterus was replaced after 20 min.

Blood collection

On the day of surgery, blood samples were collected via the jugular catheter in two phases relative to time of either replacement of the uterus for the sham-operated group or final ligation of uterine vasculature for the hysterectomized group. Blood samples were drawn at 10 min intervals during the hour preceeding replacement or ligation of the uterus (phase I). During the post-replacement or post-ligation phase (phase II), blood samples were collected at intervals of 5 min for the first 30 min, 10 min for 30 min (30 to 60 min), 30 min for 2 h (1 to 3 h) and 60 min for 2 h (3 to 5 h). Two of five cows, in the hysterectomized group, were bled at 30 min intervals during phase I and only at 1 h and 5 h during phase II. In addition, blood samples in phase III (day 1 to 15 postpartum) were collected via a jugular venipuncture twice daily (0800 and 1700 h) in the sham-operated and hysterectomized groups and once daily (0800 h) in the control group.

In each phase, blood samples were placed immediately into an ice bath; within 60 min after collection, samples were centrifuged at 5000 x g for 15 min at 4 C, and plasma stored at -20 C until assayed for 15-keto-13,14-dihydro-$PGF_{2\alpha}$ (PGFM) following modification of the method described by Eley et al. (1981b). Plasma samples were assayed for progesterone (Eley et al., 1981a) from two cows that were not ovariectomized at the time of hysterectomy and bled once or twice weekly for periods of 60 to 100 days, respectively.

Description and validation of PGFM radioimmunoassay

Plasma samples were assayed directly in duplicate without extraction or chromatography. Reaction milieu in assay tubes of experimental plasma samples was mimicked in the standard curve by the use of prostaglandin-free (PG-free) cow plasma. A pool of PG-free plasma was obtained from a day 40 postpartum cow pretreated with two 20 ml I.M. injections (1 g/injection) of Flunixin Meglumine (FM; Banamine, gift from Dr. D.A. West, Schering Corp., Kenilworth, NJ) a prostaglandin synthetase inhibitor given at 16 h intervals. The cow was bled (≈1.5 liter) 4 h after the second injection of FM, blood immediately placed in an ice bath, centrifuged within 30 min at 5000 x g for 15 min and PG-free plasma stored in 40 ml aliquots at -20 C.

PGFM standard solutions were made by serial dilutions in 0.05 M tris-HCl buffer, pH 7.5, of a stock solution (100 ug/ml in ethanol) of authentic 15-keto-13,14-dihydro-$PGF_{2\alpha}$ (gift from Dr. K. Kirton, The Upjohn Co., Kalamazoo, MI). Final PGFM standard concentrations were 0, 10, 20, 40, 70, 100, 150, 200, 300 and 400 pg/100 ul. PGFM standard curves were replicated in triplicate (10x75 mm tubes; Rochester Sci. Co., Rochester, NY) and contained 200 ul of PG-free plasma and 100 ul of a PGFM standard solution (0-400 pg/100 ul). Experimental plasma samples (200 ul maximum volume) were added to 100 ul of 0.05 M tris-HCl buffer, pH 7.5. When concentrations of PGFM in unknown samples were above the range of the standard curve, a smaller volume (25, 50 or 100 ul) was reassayed after plasma volume had been increased to 200 ul with PG-free plasma. As an additional source of precipitable protein, 100 ul of human gamma globulin (0.5 mg/100 ul) was added to each tube. Radioimmunologic reaction was performed by addition of approximately 13000 dpm of [5,6,8,11,12,14-^{3}H] PGFM (83 Ci/mM; Amersham) diluted in 200 ul of 0.5 M tris-HCl buffer, pH 7.5. A 100 ul aliquot of goat antiserum to 15-keto-13,14-dihydro-$PGF_{2\alpha}$ (gift from Dr. K. Kirton, The Upjohn Co., Kalamazoo, MI) 1:6000 dilution, was added to give a final assay volume of 700 ul. After overnight incubation at 4 C, separation of free and bound hormone was accomplished by double precipitation of proteins (plasma protein + human gamma globulin + goat anti-PGFM) with 700 ul of a cold 40% solution of polythylene glycol-6000 in water (40% PEG). Following centrifugation at 4000 x g, supernatant was discarded, pellet resuspended in 500 ul dioxane with a vortex, and scintillation cocktail (20% triton-toluene; 2.5 ml) added to each vial.

Approximately 35-40% of tritiated PGFM was bound after double precipitation of protein with 40% PEG, and nonspecific binding was less than 3.0%. Accuracy of the assay procedure was determined by measuring, in two different assays, known quantities of exogenous PGFM previously added to PG-free cow plasma. Added masses achieved final concentrations of 412, 513, 825, 1025, 1650, 2050 and 3300 pg/ml. These plasma samples were assayed in volumes of 50, 100 and 200 ul, with the exception of the highest concentrations (3300 pg/ml) which was assayed only at volumes of 50 and 100 ul. Analysis of variance was performed on measured PGFM concentrations. Main effect of assay (A), concentration (C), assay volume (V), their two (AxC; AxV; CxV) and three-way (AxCxV) interactions were tested using the General Linear Model procedure of Statistical Analysis System (SAS: Barr et al., 1979). Only the main effect of concentration was significant ($P < 0.01$). Recovery of added (X) versus measured ($\hat{Y}$) PGFM concentration was described by linear regression ($\hat{Y} = 16.38 + 0.99X$; $R^2 = 0.97$). Sensitivity of the assay was established at 50 pg/ml, since an antibody dilution of 1:6000 and an assay volume of 200 ul permitted detection of a minimum mass of

10 pg. PGFM of 10 pg significantly displaced tritiated PGFM from zero (96-98% binding; $P < 0.05$). The 16.38 pg/ml intercept (estimated by linear regression) represented a nondetectable concentration of PGFM in cow plasma that was pretreated with FM. Dilution in PG-free plasma of one low and one highly concentrated PGFM plasma samples to 0, 25 and 50% of their original concentration resulted in three inhibition curves that apparently were parallel to each other and to the standard inhibition curve (Figure 3.1; homogeneity of regression, $P > 0.1$). Ten repeated measurements of a single plasma sample among five assays resulted in intra- and interassay coefficient of variation of 8.4 and 12.4%, respectively. Crossreactivity of the antibody to PGFM with authentic $PGF_{2\alpha}$, PGE_2 and 6-keto $PGF_{1\alpha}$ was less than 1%.

Statistical analysis

For each phase (I, II and III), data were analyzed by least squares method of analysis of variance according to the General Linear Models procedure of the Statistical Analysis System (SAS; Barr et al., 1979), and polynominal response curves were used to describe time trends. Experimental design was in the nature of a split plot in which cows were nested within groups. Repeated measurements in each cow were taken over time which was considered a continuous independent variable. For each phase, group means were tested against cow within group variation. In addition, tests of homogeneity of regressions were used to test for differences in group response curves of each phase (Snedecor and Cochran, 1969).

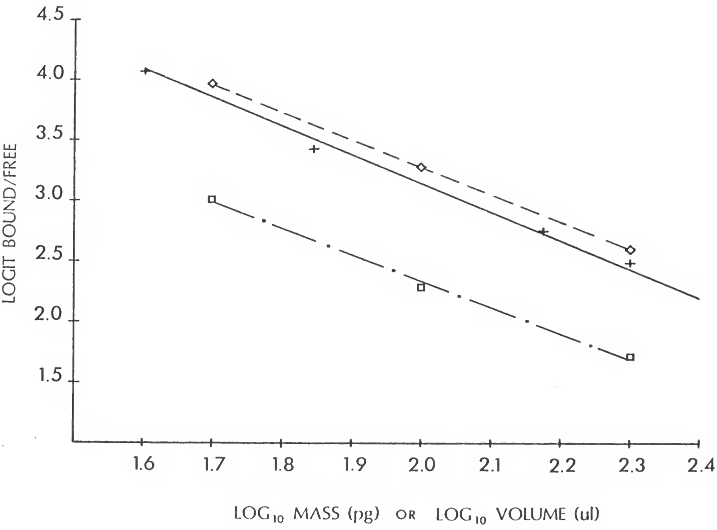


Figure 3.1 Inhibition curves of tritiated PGFM by masses of authentic radioinert PGFM (standard curve ———) and by reference plasma samples of high (— · —) and low (- - -) PGFM concentrations assayed at three different volumes (50, 100, 200 ul).

For phase III, orthogonal contrast of treatment response curves were performed.

Experiment 2

Tissue (≈ 100 cm^2) was obtained by biopsy from the midportion of the previously gravid uterine horn immediately following final ligation of uterine vasculature in three cows assigned to the hysterectomized group of Experiment 1. Uterine tissue was sealed in a plastic bag, covered with ice, and transported immediately to the laboratory. Tissue was gently washed with physiological saline (0.9% NaCl), and myometrium, caruncular and intercaruncular endometrium were obtained by dissection. Incubation of tissue as well as extraction and chromatography of arachidonic acid metabolites were described previously by Lewis et al. (1982). Briefly, each sample of uterine tissue (500 mg) was placed into a separate, sterile petri dish, sliced into pieces (≈ 3 mm x 3 mm) with scalpel blades, and 15 ml of modified minimum essential medium (MEM) added to each sample. Ten uCi of [5,6,8,9,11,12,14,15(N)-^{3}H] arachidonic acid ([^{3}H]AA; 61 Ci/mmole; New England Nuclear, Boston, MA) in 100 ul ethanol were added to the MEM in each petri dish. No radioinert arachidonic acid was added to the MEM. Immediately following addition of [^{3}H]AA, petri dishes were placed into a culture chamber (Bellco Glass Inc., Vineland, NJ) on a rocking platform (Bellco Glass Inc., Vineland, NJ) for 24 h at 37 C in an atmosphere of 50% N_2:45% O_2:5% CO_2. At the end of the incubation period, tissue and MEM were separated by centrifugation

(2000 x g) at 4 C, placed in individual glass vials, frozen immediately and stored at -20 C.

Organic solvents used for extraction and chromatography were distilled before usage. For all incubates, metabolism of [^{3}H]AA was evaluated only on products released into the medium. After double extraction of acidified MEM with ethyl acetate (Lewis et al., 1978; 1982), [^{3}H]AA and its metabolites were separated on chromatography columns containing 1.0 g of Sephadex LH-20 (Sigma Chemical Co., St. Louis, MO). Elution of material from the chromatography column was with 50 ml of a mixture of 98% methylene chloride and 2% methanol. The eluate from each column was collected into 50 tubes as 1.0 ml fractions, evaporated to dryness at 45 C and disintegrations per minute (dpm) determined in each chromatographic fraction. Calibration of chromatography columns was done by determining mobility of [5,6,8,9,11,12,14(N)-^{3}H] 15-keto-13,14-dihydro-PGF$_{2\alpha}$ ([^{3}H]PGFM; 60 to 100 Ci/mmole; Amersham Corporation, Arlington Heights, IL), (5,6,8,11,12,14,15(N)-^{3}H]PGE$_2$ ([^{3}H]PGE$_2$; 100 to 200 Ci/mmole; New England Nuclear, Boston, MA), and [9(N)^{3}H]PGF$_{2\alpha}$ ([^{3}H]PGF$_{2\alpha}$; 5 to 15 Ci/mmole; New England Nuclear, Boston, MA) in relation to mobility of [^{3}H]AA. Percentage of total dpm for each fraction from each column was calculated by dividing dpm for each fraction by total dpm recovered from the respective column. Percentage of [^{3}H]AA metabolized and released in MEM was calculated for each sample using the equation: [100-(total dpm in medium-[^{3}H]AA dpm in medium/total dpm in medium)x100]. Six regions were discerned based upon Sephadex LH-20 profiles of $[^3H]$AA metabolism by bovine caruncle tissues. Percentage of total dpm for each fraction within each region was totaled so that six percentages were derived from each column.

Data were analyzed by least squares analysis of variance according to the General Linear Models procedure of the Statistical Analysis System (SAS; Barr et al., 1979). For each region, orthogonal single degree of freedom comparisons (Snedecor and Cochran, 1969) were made to test differences between percentage of total dpm released in incubation medium of various intrauterine tissues (i.e., myometrium versus caruncular, intercaruncular endometrium; caruncular versus intercaruncular endometrium). In addition, comparison of percentages of total dpm between regions within tissue type were made by Duncan's procedure after separate least squares analysis of variance was made on each uterine tissue. Due to the limited number of cows in this experiment, cow variation was considered a fixed effect.

Experiment 3

Reproductive tracts and lung tissue, from two cows on day 2 postpartum were recovered at slaughter within 15 min of exsanguination, sealed in a plastic bag, placed on ice and transported to the laboratory. An incision was made, through the previously gravid uterine horn, and tissue was gently washed with sterile physiological saline. Pieces of myometrium, caruncular and intercaruncular endometrium were dissected, from the midportion of the previously gravid uterine horn, and 500 mg of each uterine tissue and of lung tissue (500 mg) were prepared and incubated in 15 ml MEM for 24 h under the same conditions as described previously (Experiment 2). However, $[^3H]$AA was not added. After separation of tissue and medium by centrifugation, supernatant (MEM) was transferred into a glass vial and frozen immediately at -20 C. Tissue was dried in an oven and dry weight calculated.

To quantify release of $PGF_{2\alpha}$ and PGFM into MEM by early postpartum intrauterine (myometrium, caruncular and intercaruncular endometrium) and lung tissues, acidified aliquots of medium were extracted with ethyl acetate (Lewis et al., 1978), evaporated to dryness at 40 C under a nitrogen atmosphere and rediluted in PG-free plasma. Direct radioimmunoassay of $PGF_{2\alpha}$ (Knickerbocker et al., 1984) and of PGFM (see Experiment 1) were done on extracts of MEM rediluted in PG-free plasma. The MEM extraction procedure prior to redilution in PG-free plasma did not interfere with radioimmunoassay systems of either $PGF_{2\alpha}$ or PGFM (Curl and Thatcher, unpublished observations). Mass losses were minimal during the extraction procedure; recovery of both PGFM and $PGF_{2\alpha}$ was 97%, as estimated by recovery in several samples of tritiated PGFM or $PGF_{2\alpha}$ added to medium samples selected at random.

Data from lung tissue were not considered in the statistical analyses. Responses of intrauterine tissue data were analyzed by least squares analysis of variance according to the General Linear Models procedure of the Statistical Analysis System (SAS; Barr et al., 1979). Orthogonal single degree of freedom contrasts (Snedecor and Cochran, 1969) were made to test differences in quantity of releasable PGFM and $PGF_{2\alpha}$ between uterine tissue (i.e., myometrium versus caruncular, intercaruncular endometrium; caruncular versus intercaruncular endometrium). Lung tissue was used only as a positive control to assess releasable quantity of PGFM due to its high 15-hydroxy prostaglandin dehydrogenase activity (Ferreira and Vane, 1967; Anggard et al., 1971; Maule Walker and Peaker, 1981; Hansen, 1982). Due to the limited number of animals in this experiment, cow variation was considered a fixed effect.

Results

Experiment 1

Least square means and standard errors of PGFM concentrations measured during the various phases for sham-operated, hysterectomized and control groups are presented in Table 3.1. Mean PGFM concentrations were not different ($P > 0.1$) between sham-operated and hysterectomized groups during the pre-replacement or pre-ligation phases. However, mean PGFM concentrations were lower in the hysterectomized group than the sham-operated group during the 5 h following ligation of the main uterine vasculature (phase II; 590 versus 1786 pg/ml, $P < 0.05$) and for the next 15 days postpartum (phase III; 59 versus 380 pg/ml, $P < 0.01$). Furthermore, during the chronic two week period of phase III, the control group had higher PGFM ($P < 0.01$) than the sham-operated and hysterectomized groups (980 versus 380 and 59 pg/ml, respectively).

Profiles of PGFM concentrations during the three experimental phases are best described by 5th order polynomial regression equations (Appendix A; Table A.1). During phase I (Figure 3.2), both sham-operated and hysterectomized groups had a 50% decrease in

TABLE 3.1. Least squares means (± S.E.M.) of PGFM concentrations (pg/ml) during various experimental phases in cows assigned to either a sham-operated (uterine replacement) group, hysterectomized (uterine ligation) group or control (no surgery) group.

Experimental Phases		Treatment group		
		Sham-operated	Hysterectomized	Control
I	Pre-replacement or Pre-ligation	3162 ± 846^{a}	2389 ± 555^{a}	-
II	Post-replacement or Post-ligation	1786 ± 259^{a}	590 ± 187^{b}	-
III	Days 1 to 15 postpartum	380 ± 102^{a}	59 ± 76^{b}	980 ± 114^{c}

[a,b,c]Values in row with different superscripts are different: * $P < 0.05$; ** $P < 0.01$.

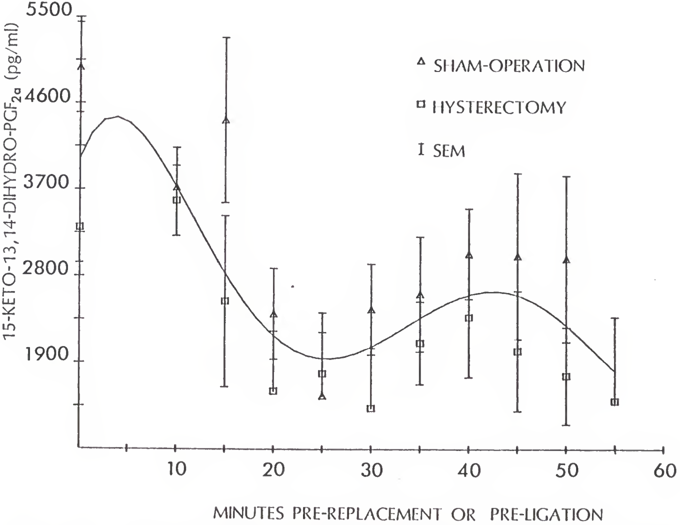


Figure 3.2 Pooled least squares regression for PGFM concentrations during the pre-replacement and pre-ligation phase in sham-operated and hysterectomized group.

PGFM concentrations, but the pattern of change did not differ (P > 0.1, Table 3.2) between groups. Although PGFM concentrations decreased in both groups following uterine replacement or ligation of the main uterine vasculature (Figure 3.3), the two curves were not parallel (P < 0.01, Table 3.2). At the beginning of phase II, PGFM concentrations were 2721 and 1700 pg/ml in the hysterectomized and sham-operated group, respectively. However, within 10 minutes following ligation of main uterine vasculature, PGFM concentrations decreased abruptly to 1442 pg/ml and further decreased to 448 pg/ml by 60 minutes. Five hours later, peripheral plasma PGFM concentrations were undetectable (PGFM <50 pg/ml) in the hysterectomized group. In contrast, PGFM concentrations for the sham-operated group averaged 2150 pg/ml during the first 2 h following uterine replacement and decreased to about 1200 pg/ml by 6 h.

During the period from day 1 to day 15 postpartum, profiles of PGFM were not parallel (P < 0.01; Table 3.2) between groups (Figure 3.4). In the control group, PGFM concentrations increased from 1154 pg/ml on day 1 to 2143 pg/ml on day 3 and decreased progressively to 110 pg/ml by day 15. In contrast, PGFM concentrations for the hysterectomized group were 90 pg/ml on day 1 and were essentially undetectable for the remainder of the postpartum period studied. Cows in the sham-operated group had a peak of PGFM of lower magnitude (1339 versus 2143 pg/ml) and duration (10 versus 15 days) than the control group. However, profile of PGFM concentrations were higher in the sham-operated

TABLE 3.2. Test of homogeneity of regression of profiles of PGFM concentrations during various experimental phases in cows assigned to sham-operated, hysterectomized and control groups.

Phase		Order of regression	Treatment		Error	
			df^b	$MS_D{}^b$	df^c	MS^c
I	Pre-replacement or pre-ligation	5	5	493540^{NS}	35	577274
II	Post-replacement or post-ligation	5	5	439758**	43	80995
III	Postpartum	5	10	1618882**,[d]	242	86682

[a] Test of homogeneity of regression between treatment: NS, not significant ($P < 0.1$); ** $P < 0.01$.

[b] Degrees of freedom and mean square (MS_D) associated with the difference in residual sums of squares between fitting one pooled curve versus an individual curve for each treatment.

[c] Degrees of freedom and mean squares associated with the error term when individual curves were generated for each treatment.

[d] Orthogonal contrast: Control versus sham-operated and hysterectomized, $P < 0.01$; sham-operated versus hysterectomized, $P < 0.01$.

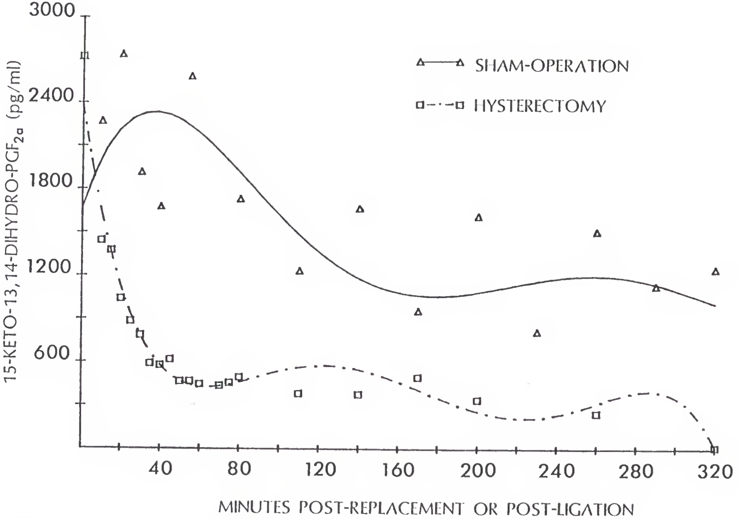


Figure 3.3 Least squares regressions and means for PGFM concentrations during the post-replacement and post-ligation phases in sham-operated and hysterectomized group, respectively.

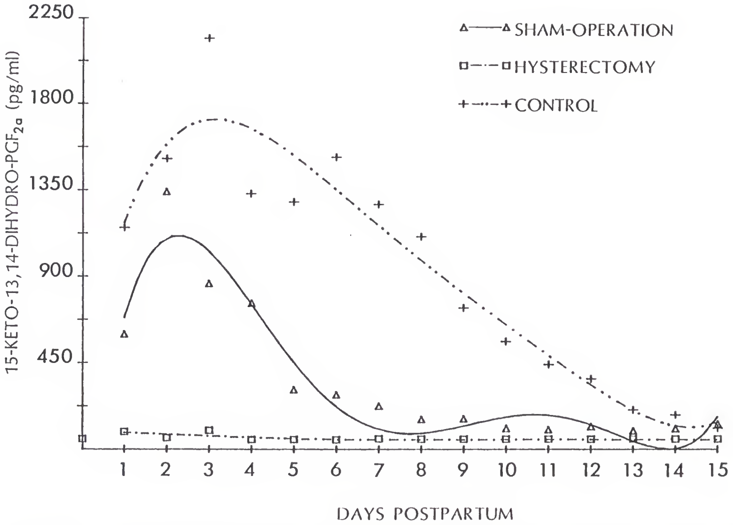


Figure 3.4 Least squares regressions and day means for PGFM concentrations from day 1 to day 15 postpartum in sham-operated, hysterectomized and control groups.

than in the hysterectomized group ($P < 0.01$, Table 3.2) for the 1 to 15 day postpartum period.

Profile of changes in PGFM concentrations in the hysterectomized group immediately after final uterine ligation (phase II, Figure 3.3), reflects the precipitous decrease in PGFM concentration for three cows bled at frequent intervals. Half life of PGFM or mean period of time needed to clear half of the initial PGFM concentration was estimated to be 18 minutes. Estimations of individual PGFM half lives for each cow were 14, 18 and 21 minutes.

Ovulation, as shown by an increase in progesterone concentration, occurred in cows that were not ovariectomized at the time of hysterectomy. Once formed, the corpus luteum (CL) was maintained throughout the subsequent blood sampling phase of each cow (Figure 3.5). An increase in progesterone concentration was first detected on day 27 in one cow, and a CL was maintained for at least 33 days. In the second animal, ovulation only occurred between day 55 and 70, and the CL also was maintained until at least day 100.

Experiment 2

Mean tissue wet weight was 496 ± 13 mg and did not differ ($P > 0.1$) between incubates of myometrium, caruncular and intercaruncular endometrium. Elution profiles for metabolism of $[^3H]$AA by bovine uterine tissues (myometrium, caruncular and intercaruncular endometrium) are illustrated in Figure 3.6. Radiolabeled compounds found in region II co-chromatographed with authentic arachidonic acid. Metabolites of $[^3H]$AA eluting in regions III, IV and V migrated with authentic 13,14 dihydro-15-keto $PGF_{2\alpha}$ (PGFM),

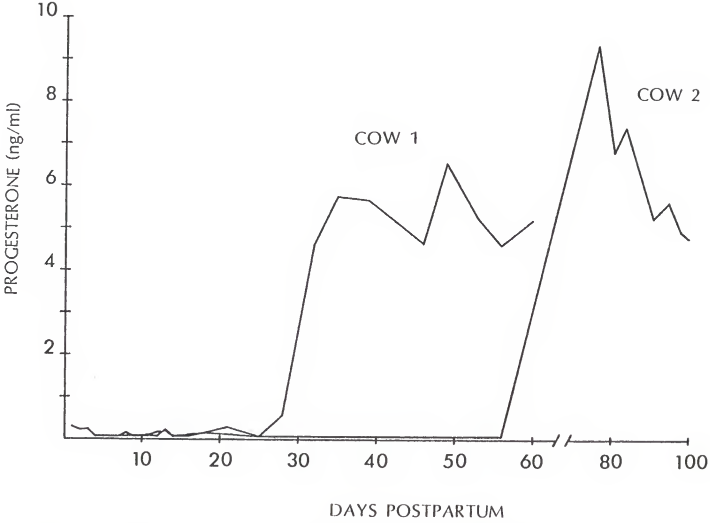
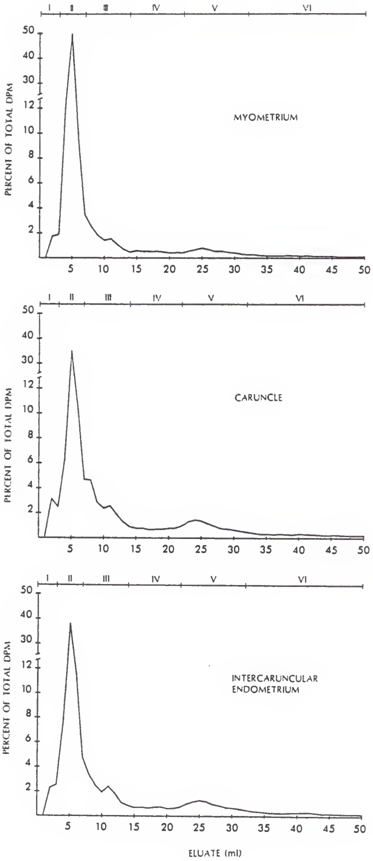


Figure 3.5 Profile of progesterone during the postpartum period in two cows not ovariectomized at the time of hysterectomy performed on the day of parturition.

Figure 3.6 Sephadex LH-20 elution profile for metabolism of $[^3H]$AA ($[^3H]$AA and its metabolites released in culture medium) after a 24 h incubation by slices of bovine myometrium, caruncular and intercaruncular endometrium collected within 8 h of parturition.


I
II
III
IV
V
VI
50
40
30
12
10
8
6
4
2
PERCENT OF TOTAL DPM
MYOMETRIUM
5
10
15
20
25
30
35
40
45
50
CARUNCLE
INTERCARUNCULAR ENDOMETRIUM
ELUATE (ml)

PGE_2 and $PGF_{2\alpha}$, respectively, whereas those found in region I and VI have not been identified. The possibility exists that other unidentified metabolites of [^{3}H]AA also migrated in to regions II, III, IV and V.

In all incubation media, the highest percentage ($P < 0.05$) of total dpm added was recovered in region II ([^{3}H]AA; Figure 3.6). Percentage of total dpm associated with metabolites of [^{3}H]AA (Table 3.3) was higher ($P < 0.05$) in region III (PGFM). Percentage dpm in region III was about two times higher ($P < 0.05$) than in region V ($PGF_{2\alpha}$). In all incubation medium, percentage of total dpm was lowest ($P < 0.05$) in regions I, IV and VI.

Percentage of [^{3}H]AA metabolized and released into the medium of myometrial incubates was 25.7 ± 2% and was lower ($P < 0.05$) than in incubation medium of caruncular (44.3 ± 4.9%) and intercaruncular endometrium (39.5 ± 5.7%). Peak values of percent of total dpm added remaining in region II [^{3}H]AA were 49.7, 34.9 and 36.4 for myometrium, caruncular and intercaruncular endometrium incubation medium, respectively (Figure 3.6). As a percentage of total dpm added and recovered from medium within regions associated with metabolites of [^{3}H]AA, medium from myometrial incubations contained less metabolites than those of caruncular and intercaruncular endometrium (Table 3.3). In all metabolite regions, percentage of dpm between caruncular and intercaruncular endometrium did not differ ($P > 0.1$). Mean percentage of total dpm in caruncular and intercaruncular endometrium incubation medium were 5.6, 2.0 and 4.1 percentage points greater

TABLE 3.3. Distribution of percent of total dpm of $[^3H]$AA in extracts of medium from incubations of bovine intrauterine tissues (myometrium, caruncular and intercaruncular endometrium) obtained by biopsy within 8 h after parturition.

	Percentage of Total dpm in Media[a]			
Region[b]	Myometrium	Caruncular Endometrium	Intercaruncular Endometrium	S.E.M.[c]
I**	3.73^d	5.72^d	4.97^d	±0.37
II (AA)**	74.95^a	57.37^a	61.73^a	±0.96
III (PGFM)**	9.43^b	16.30^b	13.92^b	±0.53
IV (PGE_2)**	3.82^c	6.16^d	5.57^d	±0.22
V ($PGF_{2\alpha}$)**	5.19^c	9.43^c	9.02^c	±0.51
VI**	2.85^d	4.99^d	4.77^d	±0.25

[a] Within a column, different superscript letters indicate differences ($P < 0.05$) among regions.

[b] For each region: orthogonal contrast: myometrium versus caruncular and intercaruncular endometrium, ** $P < 0.01$; caruncular versus intercaruncular endometrium, not significant in all regions.

[c] S.E.M. = Standard errors of the mean; they are similar for each uterine tissue within each region.

in regions III (PGFM), IV (PGE_2) and V ($PGF_{2\alpha}$), respectively, than those of medium from myometrial incubates. Likewise, in regions of unidentified metabolites of [^{3}H]AA (regions I and VI), percentage of total dpm added was lower ($P < 0.01$) in myometrium incubation medium than in medium from the two other tissue incubates.

Experiment 3

Mean quantities of immunoreactive prostaglandins ($PGF_{2\alpha}$ and PGFM), released into incubation medium by day 2 postpartum intrauterine (myometrium, caruncular and intercaruncular endometrium) and lung tissue in two cows, are presented in Table 3.4. Least squares analysis of variance of intrauterine tissue incubation medium responses is given in Table 3.5.

Regardless of type of uterine tissue, overall mean quantities of $PGF_{2\alpha}$ and PGFM measured in incubation medium were different between cows ($P < 0.01$, Table 3.5). Sum of $PGF_{2\alpha}$ and PGFM was approximately three times higher ($P < 0.01$) in one of the two animals. In both cows, mean quantities of releasable $PGF_{2\alpha}$, PGFM and their sum were lower ($P < 0.01$) in medium of myometrial incubates than in those of either the caruncular or intercaruncular endometrium. However, large differences in quantities of F series prostaglandins released by caruncular and intercaruncular endometrium incubates were measured between cows (cow x tissue interaction; $P < 0.01$; Tables 3.4 and 3.5). Quantity of $PGF_{2\alpha}$ released into incubation medium of caruncles was higher than in those of intercaruncular endometrium in only one cow and no difference in mean quantity of

TABLE 3.4. Least square means of $PGF_{2\alpha}$ and PGFM released (ng/mg dry tissue) in 15 ml of medium during a 24 h incubation by day 2 postpartum bovine intrauterine (myometrium, caruncular and intercaruncular endometrium) and lung tissues.

	Mean Quantity				
Response	Myometrium[a]	Caruncular Endometrium[a]	Intercaruncular Endometrium[a]	Lung Tissue[a,c]	S.E.M.[d]
$PGF_{2\alpha}$[b,e]	0.41	5.44	5.84	1.01	±0.45
PGFM[b,e]	0.10	3.25	1.07	6.60	±0.28
$PGF_{2\alpha}$ + PGFM[b,e]	0.52	8.70	6.92	7.62	±0.57
PGFM/$PGF_{2\alpha}$[e]	0.24	0.59	0.18	6.19	±0.02

[a] Recovered at slaughter from two cows.

[b] Significant cow x tissue interaction ($P < 0.01$; see Table 3.5).

[c] Not included in statistical analysis.

[d] Standard errors of the mean; they are similar for each tissue.

[e] Orthogonal contrast: myometrium versus caruncular and intercaruncular endometrium; for all responses, $P < 0.01$. Caruncular versus intercaruncular endometrium; $PGF_{2\alpha}$, $P > 0.1$; PGFM, $P < 0.01$; $PGF_{2\alpha}$ + PGFM, $P < 0.1$; PGFM/$PGF_{2\alpha}$, $P < 0.01$.

TABLE 3.5. Least squares analyses of variance of $PGF_{2\alpha}$ and PGFM released (ng/mg dry tissue) in 15 ml of medium during a 24 h incubation by day 2 postpartum bovine intrauterine (myometrium, caruncular and intercaruncular endometrium) tissues.

	df[a]	Mean Squares			
		$PGF_{2\alpha}$	PGFM	$PGF_{2\alpha}$+PGFM	PGFM/$PGF_{2\alpha}$
cow	1	45.62**	9.64**	97.19**	0.006
tissue[b]	2	36.60**	10.41**	74.02**	0.194**
cow x tissue	2	12.03**	5.21**	31.71**	0.002
error	6	1.04	0.05	1.51	0.002

[a] df = degrees of freedom.

[b] Lung tissue was not considered in this analysis.

** $P < 0.01$.

releasable $PGF_{2\alpha}$ could be detected between the two tissues ($P > 0.1$). Mean quantity of releasable PGFM in incubation medium was higher ($P < 0.01$) in the caruncular than in the intercaruncular endometrium in both animals although the difference between these two tissues was greater in one cow than the other. Consequently, the sum of $PGF_{2\alpha}$ and PGFM tended to be higher in the caruncular than in the intercaruncular endometrium culture medium ($P < 0.1$).

For each intrauterine tissue, ratio of $PGFM/PGF_{2\alpha}$ was similar for both cows (cow x tissue interaction; $P > 0.1$). However, differences ($P < 0.01$) in $PGFM/PGF_{2\alpha}$ ratio were detected between intrauterine tissues. Ratio of releasable $PGFM/PGF_{2\alpha}$ was higher ($P < 0.01$) in caruncular than in intercaruncular endometrium and myometrium incubation medium (0.59 versus 0.18, 0.24, respectively). In incubation medium from lung tissue, $PGFM/PGF_{2\alpha}$ ratio was approximately 20-fold higher than in intrauterine tissue incubation medium.

Discussion

Present experiments demonstrated clearly that the uterus is the primary source of F series prostaglandins ($PGF_{2\alpha}$ and/or PGFM) during the early postpartum period. Hysterectomy, performed on the day of parturition, completely eliminated the normal postpartum increase in peripheral plasma PGFM concentrations. Furthermore, bovine intrauterine tissues showed a high capacity to synthetize and release various prostaglandins during the early postpartum period. This is in agreement with in vivo (Mitchell et al., 1976; Keirse et al., 1977; Mitchell and Flint, 1978a) and in vitro

(Keirse et al., 1976; Mitchell and Flint, 1977; 1978b; Evans et al., 1982) experiments with sheep and goats during the periparturient period when concentrations of prostaglandins were increasing and the uterus was identified as an active site of prostaglandin production.

The dramatic decrease in peripheral PGFM concentrations, immediately following removal of its uterine source, resulted primarily from rapid conversion of PGFM by beta and omega oxidation steps into urinary metabolites of $PGF_{2\alpha}$. The major urinary product has been identified as $5_{\alpha},7_{\alpha}$,11-trihydroxytetranorprostane-1,16-dioic acid in cattle (Kindahl, 1980; Granstrom and Kindahl, 1982). Half-life of the parent $PGF_{2\alpha}$ compound was very short, less than one minute (Samuelsson et al., 1975) whereas half-life of its major metabolite, PGFM, was estimated to be about 8 min in humans (Samuelsson et al., 1975) and in cyclic cows (Kindahl et al., 1976). In the present experiment, half-life of PGFM averaged 18 min (range = 14-21 min) in postpartum cattle. This discrepancy might be due to a lower clearance of PGFM in the postpartum cow or to a different methodology used in estimation of the half life. In this study, some endogenous production of $PGF_{2\alpha}$ may have occurred during the process of blood clotting after manipulation and ligation of uterine vasculature. This would have contributed to an overestimation of PGFM half life. However, in a different experiment (Williams et al., 1983), half life of PGFM was estimated to be 14 min following its disappearance from peripheral blood after an intrauterine injection of $PGF_{2\alpha}$.

Massage of the uterus caused an increase in $PGF_{2\alpha}$ in the uterine vein of cyclic ewes (Roberts et al., 1975). In contrast, manipulation and prolapse of the uterus, in the present experiment, caused a reduction in peripheral PGFM concentrations prior to replacement or ligation of the uterus (Figure 3.2). Mechanical obstruction of uterine blood flow during prolapse as well as cooling of the uterus may have reduced uterine synthesis and metabolism of $PGF_{2\alpha}$ as well as their release into uterine venous blood. The possibility existed that altered uterine blood flow affected peripheral PGFM concentrations for a chronic or extended period of time since concentrations of PGFM were appreciably lower and returned to basal concentrations earlier in the sham-operated group during the 15 day period (Figure 3.4). Alternatively, lower PGFM concentrations and its earlier return to basal concentrations may reflect an alteration in the uterine prostaglandin production system following uterine prolapse.

As shown in cyclic cattle (Wiltbank and Casida, 1956; Malven and Hansel, 1964), hysterectomy performed immediately after parturition lead to CL maintenance following first ovulation in two dairy cows (Figure 3.5). This supports the concept that a uterine factor, possibly $PGF_{2\alpha}$, is involved in the process of luteolysis in cattle (Nancarrow et al., 1973; Shemesh and Hansel, 1975; Lewis et al., 1982). It also suggests that a short luteal phase, frequently observed at first ovulation (Morrow et al., 1966) may be due partly to the release of $PGF_{2\alpha}$ of uterine origin. On the other hand, first ovulation normally occurs between 14-16 days in dairy cows (Marion and Gier, 1968). Delay in time to first ovulation (27 and after 55 days) in this study may indicate the importance of the uterus in the process of resumption of early ovarian activity. Alternatively, the delay may reflect a certain degree of ovarian damage that occurred at the time of surgery.

Large in vitro production of PGFM and a greater PGFM/PGF$_{2\alpha}$ production ratio were indicative of a high capability of bovine lung tissue to synthesize and metabolize PGF$_{2\alpha}$ to PGFM. This is in agreement with previous observations in swine, sheep and goat (Ferreira and Vane, 1967; Anggard et al., 1971; Maule Walker and Peaker, 1981). However, in situ production of PGF$_{2\alpha}$ by the lung in comparison with intrauterine tissues is probably minimal since PGFM plasma concentrations were very low for the two weeks postpartum after hysterectomy.

Based upon Sephadex LH-20 column chromatography, at least five metabolites of [^{3}H]AA were found in incubation medium of various intrauterine tissues. This is at some variance with results of Lewis et al. (1982), who defined seven regions associated with metabolites of [^{3}H]AA produced by day 16 and day 19 pregnant or cyclic bovine endometrium. However, in the present experiment, although similar incubation and chromatography systems were used, separation of metabolites of [^{3}H]AA was not as refined since volume of eluant (50 ml) was collected in larger fractions (1.0 ml versus 0.5 ml). For this reason, distribution of percent dpm in region III (PGFM) was higher in this study and may reflect the presence of other unknown metabolites.

Radioactive metabolites having the same mobility as $PGF_{2\alpha}$, PGFM and PGE_2 were found in incubation medium from intrauterine tissues (Experiment 2) and presence of $PGF_{2\alpha}$ and PGFM was further confirmed by radioimmunoassay (Experiment 3). Mitchell and Flint (1977) reported that PGE_2, $PGF_{2\alpha}$ and PGFM were detectable in cotyledonary and myometrial tissues from sheep before and after labor. PGE_2 was the major prostaglandin found in the circulation of fetal lambs (Challis et al., 1976; Mitchell et al., 1980), and the fetal part of the placentome (cotyledon) was the major site of PGE_2 production in sheep (Mitchell and Flint, 1977) and in cattle (Williams and Gross, 1981). In the present experiment, metabolism of [^{3}H]AA was studied on intrauterine tissues collected within 8 h of parturition. Therefore, the possibility exists that some of the PGE_2 synthetized by bovine caruncles is related to fetal production, since interdigitated fetal and maternal microvilli of the placentome do not separate completely at parturition and caruncular tissue may be contaminated by fetal microvilli (Archbald et al., 1972). Evans et al. (1982) demonstrated the presence of 6-keto-$PGF_{1\alpha}$ (the hydrolysis product of prostacyclin) and thromboxane B_2 in fetal and maternal intrauterine tissues of the late pregnant sheep and these also may be present as metabolites in medium of intrauterine tissue incubates in the present experiment.

Finding of an appreciably lower in vitro synthesis and metabolism of prostaglandin by the postpartum bovine myometrium is consistent with previous reports in sheep (Mitchell and Flint, 1978b) and goat (Mitchell et al., 1978). $PGF_{2\alpha}$, PGFM and PGE_2

are biologically active compounds derived from arachidonic acid (Pace-Asciak, 1977). Therefore, one of the factors governing the rate of prostaglandin synthesis within a tissue would be the availability of arachidonic acid precursor. In the present study, the tendency for higher production of total F series prostaglandins ($PGF_{2\alpha}$ + PGFM) in medium by caruncular than intercaruncular endometrium incubates may reflect a higher level of endogenous arachidonic acid within this tissue. Fatty acid transfer between maternal and fetal units and arachidonic acid synthesis have been characterized recently in the ruminant and involves the placenta (Christie and Noble, 1982). The process of lipid transfer is provided by the unesterified fatty acid pool of maternal plasma and is accompanied by extensive incorporation of fatty acids into the lipid fraction of placental cotyledons (Shand and Noble, 1981). These authors also reported an increasing activity of the Δ6-desaturase enzyme system that is required for synthesis of arachidonic acid in placental cotyledons (maternal and fetal) of sheep between 120 and 150 days of gestation. Furthermore, there was a higher proportion of arachidonic acid in placental cotyledons of the sheep at term than in any of the maternal plasma lipid fractions (Noble et al., 1982). Therefore, placental cotyledons (fetal and maternal) and residual maternal placentome tissue postpartum (caruncle) are likely to be a major source of arachidonic acid substrate available for greater prostaglandin synthesis within this tissue.

A major difference between caruncular and intercaruncular endometrium tissues was in their ability to metabolize $PGF_{2\alpha}$.

Consistently more PGFM was measured in medium from caruncular incubates. In addition, as indicated by the PGFM/PGF$_{2\alpha}$ ratio, metabolism of PGF$_{2\alpha}$ to PGFM was higher in the caruncular than in the intercaruncular endometrium. This suggests a higher distribution and/or activity of 15-hydroxy prostaglandin dehydrogenase and prostaglandin Δ13-reductase (Anggard et al., 1971) in this tissue. According to Keirse et al. (1976; 1977), increased activity of the 15-hydroxy prostaglandin dehydrogenase in sheep maternal cotyledonary tissue (caruncle) as labor and parturition progressed was likely to be associated with high prostaglandin synthetic activity within the same tissue. This latter observation, together with a predominance of arachidonic acid in placentomes, would suggest that caruncular tissue is a major site for synthesis of F series prostaglandins during the early postpartum period in cattle.

Although metabolism of PGF$_{2\alpha}$ to PGFM appears to be lower in intercaruncular endometrium, this tissue might also contribute to some degree to the postpartum pool of PGFM. Perhaps some of the PGF$_{2\alpha}$ produced is being transported to the caruncle where metabolism occurs. By this mechanism, caruncles would not only produce PGF$_{2\alpha}$ but would also partially control the amount of native PGF$_{2\alpha}$ leaving the uterus.

Uterine production of prostaglandin during the early postpartum period in the cow appears to involve the caruncular tissue. In vitro synthesis, especially metabolism of PGF$_{2\alpha}$, was more noticeable in the caruncle than in other intrauterine tissues. In view of the well recognized effect of PGF$_{2\alpha}$ on smooth muscle contraction and on ovarian activity, restoration of postpartum reproductive functions may be affected by degree of $PGF_{2\alpha}$ synthesis and metabolism within the uterus. Relationships between postpartum peripheral PGFM and uterine involution have already been reported (Eley et al., 1981b; Lindell et al., 1982). Further in vivo studies are needed to establish the level of in situ production of prostaglandins within the uterus and its relationship with peripheral measurement of PGFM during the early postpartum period.

CHAPTER IV

RELATIONSHIP OF 15-KETO-13,14-DIHYDRO-PGF$_{2\alpha}$ CONCENTRATIONS IN PERIPHERAL PLASMA WITH LOCAL UTERINE PRODUCTION OF F SERIES PROSTAGLANDINS AND CHANGES IN UTERINE BLOOD FLOW DURING THE EARLY POSTPARTUM PERIOD OF CATTLE

Introduction

The early postpartum period in cattle is characterized by an elevation in 15-keto-13,14-dihydro PGF$_{2\alpha}$ (PGFM) concentrations which reached peak values on days 2 or 3 postpartum and then returned progressively to a basal concentration by day 15 (Edqvist et al., 1978; Eley et al., 1981b Lindell et al., 1982; Lewis et al., 1984). Undoubtedly, the increase in PGFM represented an uterine source of PGF$_{2\alpha}$ synthesis since hysterectomy completely eliminated this postpartum rise (see Chapter III). In addition, based on the latter study, the postpartum uterus may contribute to the peripheral concentrations of PGFM by synthesis of prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) and its metabolism in uterine caruncular tissue to PGFM. However, amount of intrauterine synthesis and metabolism of PGF$_{2\alpha}$, and the degree to which peripheral PGFM concentrations reflect the dynamic changes within the bovine uterus during the early postpartum period is unknown.

Concomitant and dose-related increases of peripheral PGFM, in response to infusions of PGF$_{2\alpha}$ into the jugular vein, suggested that measurement of PGFM in peripheral circulation provided an accurate estimate of endogenous of PGF$_{2\alpha}$ production (Kindahl et al., 1976). The assumption that peripheral measurements of PGFM concentrations reflect endogenous uterine production of $PGF_{2\alpha}$ was verified recently. An estradiol-induced increase in peripheral PGFM concentrations in cyclic or early pregnant cows (Thatcher et al., 1979; Rico et al., 1981) was accompanied temporally by an increase in content of $PGF_{2\alpha}$ in the uterine lumen (Bartol et al., 1981) and by an increase of $PGF_{2\alpha}$ synthesis and metabolism within the uterus (Knickerbocker et al., 1984).

During late pregnancy, the maternal uterine vascular bed is dilated widely (Greiss, 1971; Rosenfeld et al., 1974; 1976) and blood flow to the bovine gravid uterus (Ferrell and Ford, 1980) is much higher than during the estrous cycle and early pregnancy (Ford et al., 1979). A dramatic decrease in uterine blood flow (UBF) coincided with delivery of the feto-placental unit (Assali et al., 1958; Caton et al., 1980; Ford et al., 1982) and an apparent increase in uterine prostaglandin production. These hemodynamic changes within the uterus may affect concentrations of PGFM in the peripheral circulation by modulating rate of uterine drainage and thus delivery of $PGF_{2\alpha}$ and/or PGFM to the general circulation. Therefore, validity of a peripheral measurement of PGFM, to assess uterine synthesis and metabolism of PGFM, needs to be confirmed in view of the probable concurrent changes in UBF and uterine synthesis and metabolism of $PGF_{2\alpha}$.

Objectives of the following experiments performed during the early postpartum period in cows were to: 1) measure UBF and venous-arterial differences in $PGF_{2\alpha}$ and PGFM across the uterus and estimate their daily uterine production and 2) verify the validity of a peripheral measurement of PGFM as an estimate of uterine synthesis, metabolism and daily production of $PGF_{2\alpha}$.

Materials and Methods

Surgery

Six cows (two Holstein x Angus, three Holstein, one Jersey) were prepared for surgery within 12 h after parturition (day 0, n = 3), on day 1 (n = 1) or on day 2 postpartum (n = 1). All animals delivered healthy calves and passed their fetal membranes before surgery. At the time of surgery, cows were restrained on a portable multiple position surgery table (Douglas Tilt-Master table, Shank's Machine Co., Inc., Sterling, IL). General anesthesia was induced by rapid intravenous infusion of sodium thiamylal (3-5 g; Bio-Tal, Bio-Ceutic Laboratories, Inc., St. Joseph, MO), dissolved (12.5%) in sterile physiological saline (0.9% NaCl), which allowed introduction of a cuffed endotracheal tube into the trachea. Surgical anesthesia was maintained by constant inhalation of a mixture of oxygen and fluothane (Vescor, Orlando, FL) administered by a large animal anesthetic unit (Frazer Sweatman, Inc., Lancaster, NY). After induction of anesthesia, cows were left in an upright position and the surgery table was slightly inclined laterally. The outside posterior limb was extended backward to expose the flank region ipsilateral to the previously gravid uterine horn. Flank area was shaved and scrubbed thoroughly with germicidal soap (Medadine, Med-Tech Inc., Elwood, KS). Aseptic procedures were followed during surgery.

Uterus was exteriorized through a laparotomy incision (16-20 cm) in the paralumbar fossa. Subsequent manipulations were conducted on vasculature within the broad ligament supplying or draining the previously gravid uterine horn. Vasculature nomenclature used is the one described by Mapletoft et al. (1976). A small incision was made into the anterior leaf of the broad ligament parallel to the uterine artery (UA). Segments of a branch of uterine artery (BUA) and an uterine branch of ovarian vein (UBOV) were exposed and sterile polyvinyl catheters (0.86 mm id x 1.52 mm od; V-6; Bolab Inc., Derry, NY) inserted. A catheter in the BUA was advanced 4-6 cm into the UA. The UBOV catheter was inserted 4-6 cm with the end of the catheter remaining at least 2 cm proximal to the point of confluence between UBOV and ovarian branch of ovarian vein (OBOV). A 15-20 mm segment of UA proximal to its first bifurcation was exposed and the adventitia removed. Diameter of the vessel was estimated, and a precalibrated blood flow transducer (8-13 mm in diameter; Narco Bio-Systems Inc., Houston, TX) was placed around the UA. The lead wire close to the transducer head was sutured to the broad ligament with a series of silk sutures (Ethicon Inc., Somerville, NJ) in order to position and secure the head of the blood flow transducer around the artery. Catheters and free end-wire of the blood flow transducer were anchored to the mesometrium of the previously gravid uterine horn with silk sutures and exteriorized through the flank incision. Uterus was then reinserted into the body cavity and the incision closed. Catheters and free-end of the blood flow transducer were wrapped in gauze and stored in a reinforced pouch that was placed over the incision site and sutured to the skin. For the first 3 days following surgery, cows were given daily I.M. injections of ampicillin (Polyflex, Bristol Laboratory, Syracuse, NY).

Data Collection

To measure uterine blood flow (UBF), free-end of the blood flow transducer was connected to an electromagnetic flow meter (Narcomatic Model RT-500, Narco Bio-Systems Inc., Houston, TX), and continuous recordings were monitored with a physiograph (Desk Model DPM-4B, Narco Bio-Systems, Inc., Houston, TX). Blood flow transducers were calibrated in vitro as described previously by Roman-Ponce et al. (1978). A calibration factor for each probe was selected on the basis of obtaining a linear relationship between in vitro blood flow (X, ml/min) and output of the electromagnetic flow meter display ($\hat{Y}$, ml/min) over a range in flow from 150 to 1500 ml/min (Table 4.1). Overall differences between the two measurements was less than 5%.

On the morning following surgery (i.e., 12-24 h after surgery), cows were moved to a quiet, air-conditioned room (25 C). During periods of data collection, stress was minimal and cows were provided water ad libidum. UBF was recorded daily for a minimum of 6 h starting at 0900 h. Blood samples were collected concurrently from UA and UBOV catheters at 15 min intervals for 1 or 2 daily periods of 1 h (1000 to 1100 h and 1600 to 1700 h). Blood samples were collected into cold 5 cc glass syringes and placed immediately into an ice-bath. Samples were centrifuged (3000 x g) within 30 min of collection at 4 C, and plasma stored at -20 C until sample analysis. Between experimental days, cows were maintained in a pasture lot adjacent to the air-conditioned room and fed hay plus corn silage. Experimental data collection continued until blood flow transducers or catheters became non-functional. One cow was removed from the experiment because the blood flow transducer slipped off the UA

TABLE 4.1. In vitro calibration of blood flow transducer.

Characteristics	Transducer Size (mm)			
	8	10[a]	10	13
Regression coefficient	1.01	0.92	1.06	1.3
Intercept	-0.016	0.022	-0.008	-0.125
Correlation coefficient	0.99	0.99	0.98	0.99
Percent difference	1.0	2.2	4.9	3.8[b]
Calibration factor	100	120	150	400

[a] Used in two cows.

[b] Difference for blood flow values lower than 1000 ml/min. Tendency to overestimate for values above 1000 ml/min.

during the night following surgery. In another animal, a jugular cannula was inserted and jugular blood samples collected due to a nonfunctional UA catheter.

Prostaglandins Analyses

Plasma samples were analyzed by radioimmunoassay for 15-keto-13,14-dihydro-$PGF_{2\alpha}$ (PGFM). Description of the procedure and validation of the assay has been reported previously (see Chapter III). The antiserum (gift from Dr. K. Kirton, The Upjohn Company, Kalamazoo, MI) was prepared in goat against 15-keto-13,14-dihydro-$PGF_{2\alpha}$ conjugated at the C_1 carbon to bovine serum albumin by the carbodiimide method (Cornette et al., 1974). Unextracted plasma samples were assayed and separation of free and bound radiolabeled PGFM was done by protein precipitation with polyethylene glycol-6000. Twenty repeated measurements of a reference sample in eight assays gave intra- and interassay coefficients of variation of 9.5% and 3.7%, respectively.

Concentrations of $PGF_{2\alpha}$ in unextracted plasma samples were measured with the double radioimmunoassay system described by Cornette et al. (1972). Procedure of this assay has been reported and validated elsewhere (Moeljono et al., 1977; Bartol et al., 1981) for pig plasma samples and bovine uterine flushings. First antibody (gift from Dr. K. Kirton, The Upjohn Company, Kalamazoo, MI) was generated in rabbits against $PGF_{2\alpha}$ conjugated to bovine serum albumin at the C_1 carbon. The second antibody (Cappel Laboratories Inc., Dowington, PA) was produced in a goat against both heavy and light chains of IgG purified from rabbit serum. Assay procedure for cow plasma was modified slightly. Reaction milieu in assay tubes of unknown plasma samples was mimicked in the standard curve by the use of prostaglandin-free (PG-free) cow plasma (see Chapter III). In addition, a maximum plasma volume of 200 ul was assayed and mass of $PGF_{2\alpha}$ in tubes of the standard curve ranged from 10 to 1000 pg. Precision of the assay was evaluated by adding masses of $PGF_{2\alpha}$ to PG-free plasma that achieved final concentrations of 291, 582, 625, 1165, 1250 and 2500 pg/ml. Spiked samples were assayed at volumes of 50, 100 and 200 ul. Mass recovered ($\hat{Y}$) was described by linear regression ($\hat{Y} = -13.09 + 1.06X$; $R^2 = 0.95$) and concentrations measured were not affected ($P > 0.1$) by the volume assayed. Sensitivity of the assay was established at 50 pg/ml since a minimum mass of 10 pg could be detected and maximum volume assayed was 200 μl. Dilutions in PG-free plasma of plasma samples collected in UBOV of postpartum cows, to 0, 25, and 50% of their original concentrations (1210 ± 48 and 2528 ± 254 pg/ml, respectively) resulted in two inhibition curves that apparently were parallel to each other and to the standard inhibition curve (homogeneity of regression, $P > 0.1$). Intra- and interassay coefficient of variations of reference plasma samples collected in the UBOV during the early postpartum period were 11.3% and 2.2%, respectively.

Physiological Responses

Plasma concentrations of $PGF_{2\alpha}$ or PGFM, for each arterial and venous blood sample, were considered as an estimate of the respective prostaglandin concentrations in uterine arterial affluent (UA) and venous effluent (UBOV) of the previously gravid uterine horn. For each pair of simultaneous samples within a day, venous-arterial (V-A) differences of $PGF_{2\alpha}$ and PGFM as well as their sum (V-A, $PGF_{2\alpha}$ +

V-A, PGFM) were calculated. Similarly, ratio of venous to arterial (V/A) concentrations of each prostaglandin and ratio of V-A differences of $PGF_{2\alpha}$ to PGFM (V-A, $PGF_{2\alpha}$/V-A, PGFM) were obtained. A daily estimate of each of these prostaglandin responses was obtained after observation values within a day were pooled statistically.

A uterine blood flow measurement was obtained from the average of 15 consecutive values taken at 1 min intervals during the period preceeding blood collection from UA and UBOV catheters. This response was considered as an estimate of arterial blood flow to the previously gravid uterine horn at the time paired blood samples were withdrawn. A daily estimate of arterial blood flow to that horn was obtained by averaging the 15 min blood flow values associated with each blood sampling period within a day.

In situ daily uterine production (mg/day) of prostaglandin ($PGF_{2\alpha}$, PGFM, and $PGF_{2\alpha}$ + PGFM) was estimated from the mean UBF and V-A differences as mg/day = [(V-A differences, pg/ml) x (UBF, ml/min/horn) x (1440 min/day) x (2 horns)]. Assumption that blood flows are equal between previously gravid and nongravid uterine horns undoubtedly leads to overestimations of UBF and prostaglandin production rates since measurements were only taken from the larger previously gravid uterine horn.

Statistical Analyses

Surgical preparations remained functional until day 14 and day 9 postpartum in two cows. However, for statistical analyses, only the period from day 1 to day 5 postpartum was considered since a more balanced data set across all cows was available during this period (Table 4.2). Data were analyzed by least squares analysis of variance utilizing the General Linear Models procedure of the mathematical model included variation for cow and day was a discrete independent variable. Statistical Analysis System (SAS; Barr et al., 1979). Orthogonal single degree of freedom comparisons (Snedecor and Cochran, 1969) were made to test day means. Since UBF varied during the postpartum period of blood sampling, statistical analysis of selected prostaglandin responses also included UBF as a covariate. No attempts were made to characterize time trends within a day. Interrelationships of UBF, V-A differences in $PGF_{2\alpha}$ and PGFM and arterial PGFM measurements were evaluated utilizing gross-among cow and partial-within cow correlations during the postpartum experimental period.

Results

Daily changes in mean UBF to the previously gravid horn, during the early postpartum period, are depicted in Figure 4.1. UBF averaged 3079 ± 683 ml/min on day 1 postpartum and decreased by approximately 80.5% to 602 ± 56 ml/min on day 2 and to 494 ± 184 ml/min on day 3 ($P < 0.05$). Decreases in UBF were less dramatic over the next 2 days and averaged 396 ml/min on days 4 and 5.

Concentrations of both $PGF_{2\alpha}$ and PGFM were higher ($P < 0.01$) in the UBOV than in the UA (Table 4.3). Over the entire experimental period, mean $PGF_{2\alpha}$ concentrations in the UBOV were 4.64 ± 0.6 ng/ml, whereas they were frequently undetectable in the UA and averaged only 81 ± 20 pg/ml. During the same period, mean PGFM concentrations were 5.67 ± 0.41 and 1.02 ± 0.09 ng/ml in the UBOV and UA, respectively.

TABLE 4.2. Number of cows, blood samples (venous, arterial and paired venous-arterial) and uterine blood flow (UBF) observations during the first 5 days postpartum.

	Days Postpartum				
Number of	1	2	3	4	5
Cows	2	5[a]	5	4	3
Venous (V) samples	15	35	22	18	6
Arterial (A) samples	15	35	18	21	5
Paired V-A samples	15	35	16	18	5
UBF observations[b]	15	19[c]	29	30	14

[a] Blood sampling was started within 10 h of surgery in one animal.

[b] Each observation was derived from the average of the 15 consecutive 1 min intervals UBF values recorded prior to each blood sample collection.

[c] UBF not recorded for three animals during that day.

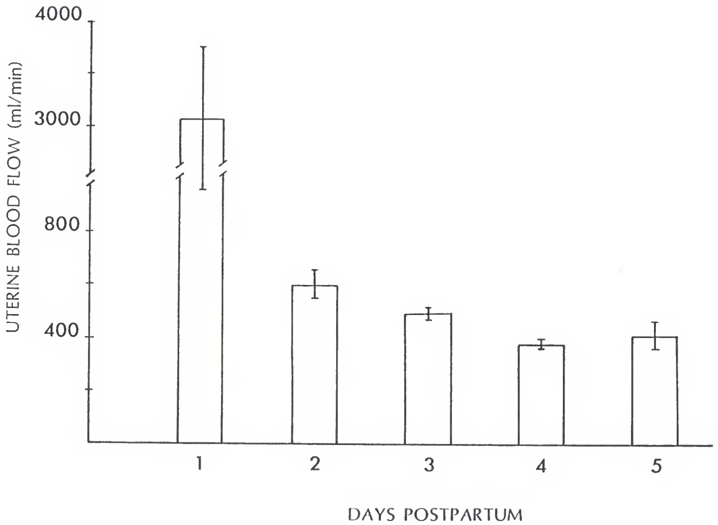


Figure 4.1 Changes in uterine blood flow to the previously gravid uterine horn during the early postpartum period in cows (X ± S.E.M.).

Concentrations of $PGF_{2\alpha}$ in the UA were low (< 125 pg/ml) throughout the entire experimental period and there were no detectable day trends ($P > 0.1$). Meanwhile, mean PGFM concentrations in the UA were 1.53 ± 0.2 ng/ml on day 1 and decreased ($P < 0.05$) progressively to an average concentration of 0.60 ng/ml on days 4 and 5 postpartum.

Mean concentrations of $PGF_{2\alpha}$ in the UBOV were 3.76 ± 1.5 ng/ml on day 1 and increased ($P < 0.1$) by nearly 60% between days 2 and 3 (Table 4.3). Very high concentrations of $PGF_{2\alpha}$ in the UBOV were measured in 1 of 3 cows on day 5 ($PGF_{2\alpha}$ > 20 ng/ml). This precluded detection of changes ($P > 0.1$) during the last 2 days of the experimental period despite the apparent decrease in venous concentrations of $PGF_{2\alpha}$ that was observed on day 4. Venous-arterial (V-A) differences in $PGF_{2\alpha}$ (paired samples only) were constant ($P > 0.1$) during the first 3 days postpartum and averaged 4.03 ng/ml. Subsequently, V-A differences in concentration of $PGF_{2\alpha}$ reflected concentration of $PGF_{2\alpha}$ in the UBOV. Concentrations of PGFM in the UBOV ($P < 0.1$) and V-A differences in PGFM ($P < 0.05$) peaked on days 2 and 3 and then decreased ($P < 0.05$ and $P < 0.1$, respectively) to levels that were comparable to those measured on day 1. Venous-arterial differences in total F series prostaglandins ($PGF_{2\alpha}$ and PGFM) were maintained at an average value of 8465 pg/ml during the first 3 days postpartum. Due to large variation in $PGF_{2\alpha}$, changes that occurred in total F series prostaglandins during days 4 and 5 were not significant ($P > 0.1$).

After adjustment for changes in UBF by covariate analysis, changes in PGFM concentrations in the UA persisted ($P < 0.01$)

TABLE 4.3. Least squares means (± SEM) of $PGF_{2\alpha}$ and PGFM concentrations in uterine arterial (UA) affluent and venous (UBOV) effluent and their venous-arterial (V-A) differences during the early postpartum period in cows.

	Days Postpartum					Orthogonal Contrasts[b]			
Response (pg/ml)	1	2	3	4	5	A	B	C	D
UA Concentrations (A)									
$PGF_{2\alpha}$, pg/ml	51±50	89±32	123±45	50±44	78±86	NS	NS	NS	NS
PGFM, ng/ml	1.53±0.2	1.27±0.1	0.84±0.2	0.47±0.2	0.73±0.4	*	**	*	NS
PGFM, ng/ml[a]	2.10±0.3	1.48±0.2	0.68±0.2	0.30±0.2	0.67±0.4	**	**	**	NS
UBOV Concentrations (V)									
$PGF_{2\alpha}$, ng/ml	3.76±1.5	4.45±1.0	7.11±1.2	1.37±1.3	8.68±2.4	NS	NS	+	*
PGFM, ng/ml	4.89±1.0	6.02±0.6	7.59±0.8	3.40±0.9	4.53±1.6	+	**	NS	NS
PGFM, ng/ml[a]	6.54±1.3	6.86±0.9	7.23±0.8	2.98±0.9	4.50±1.8	NS	**	NS	NS
V-A Differences									
$PGF_{2\alpha}$, ng/ml	3.71±1.3	4.36±0.9	4.01±1.3	1.30±1.2	6.20±2.3	NS	NS	NS	*
PGFM, ng/ml	3.36±0.7	4.75±0.5	5.25±0.7	3.12±0.7	3.09±1.5	*	+	NS	*
$PGF_{2\alpha}$ + PGFM, ng/ml	7.07±2.0	9.11±1.3	9.20±2.0	4.4±1.8	10.8±3.8	NS	NS	NS	*

[a] UBF = Significant covariate, $P < 0.05$.

[b] Orthogonal contrasts for day means: A = 1 vs 2, 3 C = 2 vs 3
B = 1, 2, 3 vs 4, 5 D = 4 vs 5.

**, $P < 0.01$; *, $P < 0.05$; +, $P < 0.1$; NS = not significant, $P > 0.1$.

although absolute mean value may have changed (Table 4.3). Concentrations of PGFM ($P < 0.05$) but not of $PGF_{2\alpha}$ ($P > 0.1$) in the UBOV were affected by changes in UBF. After adjusting for UBF as a covariate, PGFM concentrations in UBOV no longer showed a rise during days 2 and 3. Instead, mean concentrations of PGFM were higher on day 1 (after UBF adjustment), remained elevated until day 3 and then decreased ($P < 0.01$).

Two factors contributing to variation in ratio of uterine venous to arterial PGFM were the degree of metabolism of $PGF_{2\alpha}$ within the uterus and changes in peripheral concentrations of PGFM. Prior to adjustment for changes in UBF, ratio of venous to arterial concentrations of PGFM (Table 4.4) increased ($P < 0.01$) from day 1 to day 5 but this trend was not significant ($P > 0.1$) after adjusting for UBF as a covariate. This indicated that changes in UBF altered intrauterine metabolism of $PGF_{2\alpha}$ and/or peripheral PGFM concentrations. On the other hand, ratio of V-A differences in PGFM to $PGF_{2\alpha}$, which provided an estimate of the relative degree of intrauterine synthesis and metabolism of $PGF_{2\alpha}$, did not vary significantly ($P > 0.1$) during the period studied and was not affected by variations in UBF. This suggests that the degree of metabolism of $PGF_{2\alpha}$ parallels its degree of synthesis within the uterus.

Estimates of daily uterine production of F series prostaglandins, calculated from V-A differences and UBF, are presented in Table 4.5. Production of PGFM decreased from 15.2 ± 1.7 to 3.4 ± 1.60 on day 4 ($P < 0.01$) and then remained stable ($P > 0.1$). Production of $PGF_{2\alpha}$ and of total F series prostaglandins ($PGF_{2\alpha}$ and PGFM) also decreased during the first 4 days but large concentrations of $PGF_{2\alpha}$ measured

TABLE 4.4. Daily mean ratios of venous (UBOV) to arterial (UA) concentrations of PGFM and ratios of V-A differences in PGFM to V-A differences in $PGF_{2\alpha}$ during the postpartum period of cows.

Ratio	Days Postpartum					Day Trends[a]
	1	2	3	4	5	
PGFM, UBOV/PGFM, UA						
not adjusted for UBF	4.30±0.61	4.36±0.34	7.89±0.56	9.14±0.58	8.69±1.02	L
adjusted for UBF[b]	4.87±0.81	6.80±0.64	6.05±0.72	6.72±0.84	7.64±1.19	NS
V-A, PGFM/V-A, PGF						
not adjusted for UBF	2.53±0.28	2.75±0.15	3.51±0.25	3.13±0.25	2.86±0.44	NS
adjusted for UBF[b]	3.40±0.39	3.35±0.30	2.83±0.36	2.28±0.40	2.39±0.53	NS

[a] L = linear day trend, $P < 0.01$; NS = no significant day trend, $P > 0.1$.
[b] UBF utilized as a covariate.

TABLE 4.5. Estimates of daily production of F series prostaglandins during the early postpartum period.

Production (mg/day)[a]	Days Postpartum					Orthogonal Contrasts[b]			
	1	2	3	4	5	A	B	C	D
$PGF_{2\alpha}$[c]	13.3±3.0	13.0±2.7	6.9±2.9	1.4±2.8	13.3± 5.9	NS	NS	NS	NS
PGFM	15.2±1.7	12.1±1.6	8.3±1.7	3.4±1.6	4.8± 3.9	*	**	+	NS
$PGF_{2\alpha}$ + PGFM[c]	28.5±4.5	25.1±4.0	14.9±4.6	4.9±4.3	22.5±10.2	NS	NS	NS	NS

[a] Daily production = V-A differences (pg/ml) x UBF (ml/min/horn) x 1440 min/day x 2 horns.
[b] See Table 4.3.
[c] Significant decrease ($P < 0.01$) from days 1 to 4 when data from 1 of 3 cows on day 5 are omitted.
**, $P < 0.01$; *, $P < 0.05$; +, $P < 0.1$; NS = not significant, $P > 0.1$.

in UBOV of 1 of 3 cows on day 5 invalidated the statistical significance of this decrease. When data from this animal on day 5 are omitted, decrease in production of $PGF_{2\alpha}$ and of total F series prostaglandins between days 1 and 4 postpartum is significant ($P < 0.01$).

Decreases in UBF were not associated with uterine venous concentrations or V-A differences in $PGF_{2\alpha}$ or PGFM ($P > 0.1$; Table 4.6). Local V-A differences in PGFM across the uterus were correlated positively ($P < 0.01$) with venous concentrations, V-A differences and daily production of $PGF_{2\alpha}$. Similarly, concentrations of PGFM in the UA, which are equivalent to peripheral samples also were correlated (Table 4.6) positively ($P < 0.01$) with uterine venous and V-A differences in $PGF_{2\alpha}$, PGFM and of total F series prostaglandins ($PGF_{2\alpha}$ + PGFM). Furthermore, there was a positive correlation ($P < 0.01$) between PGFM concentrations in the uterine artery and estimates of daily uterine production of $PGF_{2\alpha}$ and total F series prostaglandins ($PGF_{2\alpha}$ + PGFM).

Discussion

As evidenced by significant positive correlations in the present study, measurement of PGFM in the uterine artery or an equivalent peripheral sample, is a good indicator of local uterine V-A differences in F series prostaglandins as well as a relative index of daily production of these prostaglandins in postpartum cows. This is in agreement with results of Knickerbocker et al. (1984), indicating that peripheral measurements of PGFM were associated with uterine $PGF_{2\alpha}$ production in cyclic cows.

TABLE 4.6. Within cow partial correlations of uterine blood flow, V-A differences in PGFM and PGFM concentrations in uterine artery with various uterine hormonal responses and uterine blood flow.

	Within Cow Partial Correlations		
Response	Uterine blood flow	PGFM V-A differences[a]	PGFM concentrations in uterine artery[a]
UBOV concentrations			
$PGF_{2\alpha}$	NS	0.46**	0.48**
PGFM	NS	-	0.51**
V-A differences			
$PGF_{2\alpha}$	NS	0.46**	0.49**
PGFM	NS	-	0.42**
$PGF_{2\alpha}$ + PGFM	NS	-	0.53**
Daily production			
$PGF_{2\alpha}$	-	0.31**	0.37**
PGFM	-	-	NS
$PGF_{2\alpha}$ + PGFM	-	-	0.33**

[a] Gross among cow correlation coefficients were greater than 0.80, $P < 0.01$.

**, $P < 0.01$; +, $P < 0.1$; NS = not significant, $P > 0.1$.

Large and sustained V-A differences in both $PGF_{2\alpha}$ and PGFM concentrations were indicative of a high degree of synthesis and metabolism of $PGF_{2\alpha}$ within the bovine uterus during the early postpartum period. Results agree with previous findings demonstrating that postpartum intrauterine tissue had the capability to synthesize and metabolize $PGF_{2\alpha}$ and that the uterus was the main tissue contributing to the postpartum increase in F series prostaglandins production as measured by concentrations of PGFM in peripheral plasma (see Chapter III). On a daily basis, estimates of endogenous production rates of $PGF_{2\alpha}$ by the uterus (Table 4.5) are in agreement with those measured indirectly by Kindahl et al. (1982). Results of the present experiment demonstrated that daily production of F series prostaglandins decreased progressively (Table 4.5). Since continual high levels of in utero prostaglandin synthesis (as reflected by positive V-A differences) prevailed during the early postpartum period (Table 4.3), decreases in UBF may be the main factor associated with decrease in estimates of daily production of F series prostaglandins.

The dramatic decrease in UBF between days 1 and 2 postpartum and the progressive decline thereafter is in general agreement with previous observations in the cow (Ford et al., 1982) and sheep (Assali et al., 1958; Caton et al., 1976; 1980). The postpartum decline in UBF (Figure 4.1) was delayed by approximately 1 day compared to the trend described by Ford et al. (1982). This difference may be due to surgery being performed after parturition in the present study versus prepartum in the latter.

Mechanisms of control of utero-placental blood flow have not been elucidated. In cattle, increases in UBF during gestation coincide with increases in estrogens produced within the gravid uterus, but maintenance of high rates of UBF (above 3000 ml/min) during the last trimester of gestation appears to be independent of these steroids (Ferrell and Ford, 1980). Near term, approximately 80% of total UBF in sheep is distributed to maternal part of the placentomes (Makowski et al., 1968; Rosenfeld et al., 1974); the maternal placental vascular bed, even at the level of distal caruncular arterioles (Rosenfeld et al., 1976), is kept near maximal vasodilation (Greiss and Anderson, 1974). Therefore, an acute decrease in UBF at parturition is most likely due to an increase in vascular resistance as a result of vasoconstriction within the maternal placental vascular bed. According to Rankin and McLaughlin (1979), maternal placental blood flow does not appear to be under maternal control, and accumulating evidence indicates that prostaglandins of conceptus origin may be the controlling factors which regulate utero-placental vasculature (Terragno et al., 1974; Rankin, 1976; Rankin and McLauglin, 1979; Clark et al., 1982). Fetal vessels produce prostacyclin (PGI_2; Terragno et al., 1978), which is a potent vasodilator of cotyledonary vasculature, and is likely to be involved in maintenance of UBF (Clark et al., 1982). Therefore, it is possible that the decrease in UBF after parturition is due to removal of a vasodilatory factor, perhaps PGI_2, along with expulsion of fetus and placenta. This agrees with the previous observation that UBF remained high until delivery of the fetus and expulsion of the placenta in cattle (Ford et al., 1982). Absence of a relationship between uterine venous concentrations of $PGF_{2\alpha}$, a known vasoconstrictor (Rankin and McLauglin, 1979; Clark et al., 1982) and UBF (Table 4.6) would suggest that a reduction in postpartum UBF is more dependent upon removal of a vasodilatory substance than upon synthesis of a vasoconstrictor such as $PGF_{2\alpha}$. However, other vasoconstrictor agents such as catecholamines (Barton et al., 1974; Anderson et al., 1977) may modulate maternal uterine vascular resistance and should not be excluded. Postpartum changes in UBF were correlated with changes in progesterone and estrogens in sheep (Caton et al., 1980) but not in cattle (Ford et al., 1982).

Extent of synthesis and metabolism of $PGF_{2\alpha}$ within the uterus was unknown during the early postpartum period since only peripheral PGFM (Edqvist et al., 1978; Eley et al., 1981b; Lindell et al., 1982; see Chapter III) or uterine venous $PGF_{2\alpha}$ concentrations (Fairclough et al., 1975) were evaluated. In comparison with venous-arterial differences of F series prostaglandins measured across the uterus of cows during late gestation (Gimenez et al., 1983), those measured across the bovine uterus during the postpartum period were several times higher. Therefore, in association with expulsion of the feto-placental unit, an increase in intrauterine activities of phospholipase A_2 and cyclo-oxygenase were undoubtedly responsible for $PGF_{2\alpha}$ synthesis (Ramwell et al., 1977) with both $PGF_{2\alpha}$ and PGFM being released as major products in the cow. According to Poyser (1981), increased release of prostaglandins by the uterus is due to increased synthesis since prostaglandins are not stored in tissues.

Changes in arterial UBF are reflected in uterine venous blood flow (Christenson and Prior, 1978). Thus, increase in concentrations of $PGF_{2\alpha}$ in the UBOV during the early postpartum period (until day 3) may partially reflect an increase in concentration that is associated with the decrease in UBF. However, as changes in UBF did not affect concentrations of $PGF_{2\alpha}$ in the UBOV, increases in $PGF_{2\alpha}$ on days 2 and 3 postpartum were due likely to an increased synthesis.

Extensive metabolism of $PGF_{2\alpha}$ during the early postpartum period in cows did not appear to occur at the expense of a pool of $PGF_{2\alpha}$ in the uterus. Results of this study indicated that metabolism was rather dependent upon continual synthesis of $PGF_{2\alpha}$ since V-A differences in $PGF_{2\alpha}$ and PGFM concentrations were correlated positively ($r = 0.46$). In addition, ratio of V-A differences in PGFM to $PGF_{2\alpha}$ concentrations was constant throughout the postpartum period studied and was not influenced by variations in UBF (Table 4.4). In response to estradiol-induced increases in $PGF_{2\alpha}$ synthesis, metabolism of $PGF_{2\alpha}$ to PGFM within the uterus was stimulated in the cyclic cow (Knickerbocker et al., 1984). Collectively, these results suggest that the enzymatic system (15-hydroxy prostaglandin dehydrogenase and prostaglandin Δ-13 reductase; Anggard et al., 1971) required to metabolize $PGF_{2\alpha}$ is gradually stimulated within the uterus as the level of synthesis increases.

Intrauterine metabolism of $PGF_{2\alpha}$ appeared to be modulated to some extent by changes in UBF. Increases in the ratio of uterine venous to arterial PGFM and in mean PGFM concentrations in the UBOV (with UBF as covariate) on day 1 may reflect an increase in metabolism of $PGF_{2\alpha}$ as a consequence of an increased retention time of $PGF_{2\alpha}$ within the uterus when UBF decreased. Alternatively, increasing ratio of uterine venous to arterial PGFM concentrations may represent decreasing concentrations of peripheral or arterial PGFM as a result of decreasing uterine drainage when UBF decreased. After adjustment for changes in UBF, the ratio of uterine venous to arterial PGFM concentrations was constant throughout the period studied. This suggests that at a constant UBF, uterine venous and arterial PGFM concentrations are directly proportional during the early postpartum period in cattle.

Ability of early postpartum caruncular endometrium to synthesize $PGF_{2\alpha}$ has been demonstrated and capability of this tissue to metabolize $PGF_{2\alpha}$ was greater than the myometrium or the intercaruncular endometrium (see Chapter III). Keirse et al. (1976) suggested that high metabolism of $PGF_{2\alpha}$ by the caruncle reflected the degree of synthetic activity within this tissue. Therefore, it is possible that output of F series prostaglandins from the uterus originate mainly from synthesis and metabolism of $PGF_{2\alpha}$ within the caruncles. Inhibition of prostanoid production by bovine caruncular endometrial cells during gestation (Shemesh et al., 1984) was associated with the presence in term bovine fetal cotyledon of a cytosolic protein that is a potent inhibitor of prostaglandin synthetase (Shemesh et al., 1981). Removal of fetal cotyledonary inhibition at parturition (delivery of calf and fetal placenta) would likely increase production of $PGF_{2\alpha}$ and its subsequent metabolism by the richly vascularized maternal caruncles. Capability of bovine arteries and veins to synthesize $PGF_{2\alpha}$, the parent compound, was demonstrated previously

(Terragno et al., 1978). Near term, content of arachidonic acid within the placentome (fetal cotyledons and maternal caruncles) is higher than in any other maternal plasma lipid fractions (Noble et al., 1982). Furthermore, Lindell et al. (1982) reported that increase in peripheral PGFM concentrations only occurred when abortion was induced in cows that were pregnant for more than 100 days. At this time, placentome formation is completed and continued caruncular growth occurs throughout pregnancy (Hammond, 1927; Hutchinson, 1962). Thus, bovine caruncular tissue is likely to be a major source of large quantities of F series prostaglandins measured in uterine venous blood during the early postpartum period.

It was shown in the present experiment that a peripheral measurement of PGFM is a good index of intrauterine production and metabolism of $PGF_{2\alpha}$. Furthermore, evidence implicates the caruncle as a major site of synthesis and metabolism of $PGF_{2\alpha}$ during the early postpartum period in cattle. Species with a cotyledonary-caruncular attachment are classified as delayed deciduous since maternal caruncular tissue is lost eventually during the postpartum period but not at parturition. In cattle, caruncular arteries constrict and caruncular blood supply decreases during the first 10 days postpartum (Kiracofe, 1980). In contrast, in the pig the entire placenta which is classified as diffuse and nondeciduous (Steven, 1975) is delivered at parturition. In cattle, persistence of high concentrations of peripheral PGFM and their gradual decline during the early postpartum period may be due to persistence of caruncular tissue for some time after parturition. This may reflect the degree of caruncular growth and/or vascularization in response to feto-maternal interactions during gestation. Therefore, it is proposed that postpartum peripheral concentrations of PGFM are a functional index of the maternal part of the placentome.

CHAPTER V
HORMONAL, UTERINE AND OVARIAN RESPONSES TO PHYSIOLOGICAL INFUSIONS OF $PGF_{2\alpha}$ IN POSTPARTUM COWS WITH SUPPRESSED ENDOGENOUS PRODUCTION OF PROSTAGLANDINS

Introduction

After delivery of the feto-placental unit, the postpartum bovine uterus enters an involutionary phase that ultimately leads to restoration of a suitable environment for establishment of a new pregnancy. Morphological and histological changes of the involuting uterus have been described for the postpartum cow (Casida, 1968; Morrow et al., 1966; Gier and Marion, 1968; Wagner and Hansel, 1969) and were reviewed by Kiracofe (1980). Drastic reduction in uterine size, tissue loss and tissue repair were characterized as overlapping processes involved in uterine involution (Gier and Marion, 1968). Estimations of time intervals from parturition to complete uterine involution, as assessed by rectal palpation varied greatly. Estimates ranged from 28 to 56 days among studies reviewed by Casida (1968) and Kiracofe (1980) and averaged 34 and 40 days in primiparous and pluriparous cows, respectively (Marion et al., 1968).

As the uterus involutes, considerable ovarian follicular activity occurs as evidenced by the constant presence of nearly mature follicles soon after parturition (Foote and Peterson, 1968; Marion and Gier, 1968; Saiduddin et al., 1968; Wagner and Hansel, 1969; Stevenson et al., 1983). Recent histological findings further substantiate this process in that the large early postpartum pool of small antral follicles was depleted rapidly to favor replenishment of classes of larger antral follicles (Dufour and Roy, 1983). First ovulation usually occurred within the first 3 weeks in the majority of postpartum dairy cows (Marion and Gier, 1968; Stevenson and Britt, 1980) and preferentially involved ovaries contralateral to the previously gravid uterine horn (Marion and Gier, 1968; Foote and Peterson, 1968; Casida, 1968). In comparison with ovulation in cyclic cow, the corpus luteum (CL) was smaller in size and had a shorter life span after first ovulation pospartum (Menge et al., 1962; Marion and Gier, 1968). This was also associated with lower plasma progesterone concentrations (Kesler et al., 1978; Stevenson and Britt, 1979; Ward et al., 1979). Therefore, ovulation and luteal function postpartum may be dependent upon previous conditions of intra-ovarian follicular development that may alter responsiveness of developing follicles to gonadotropins.

The early postpartum uterus produces large amount of F series prostaglandins (see Chapter III and IV) that are reflected by high peripheral concentrations of 15-keto-13,14-dihydro-PGF$_{2\alpha}$ (PGFM; Edqvist et al., 1978; Eley et al., 1981b; Lindell et al., 1982). Longer duration (Lindell et al., 1982) and higher magnitude (Lewis et al., 1984) of postpartum peripheral plasma PGFM concentrations have been related to shorter intervals for completion of uterine involution and may favor an earlier resumption of ovarian activity (Marion and Gier, 1968). Furthermore, prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) may affect gonadotropin release from the pituitary (Haynes et al., 1978; Bono et al., 1980) and be involved directly in follicular development (Armstrong, 1981; Tam and Roy, 1982). In Chapter IV, measurements of uterine blood flow and concentration gradients of $PGF_{2\alpha}$ and PGFM across the postpartum uterus provided daily estimates of uterine production for F series prostaglandins. Therefore, objectives of this experiment were to determine the role of $PGF_{2\alpha}$ in the processes of uterine involution and resumption of ovarian activity. This was accomplished by infusing physiological doses of $PGF_{2\alpha}$ in postpartum cows whose endogenous production of prostaglandins was suppressed and conducting a replacement therapy type of experiment.

Materials and Methods

Animals and Surgery

On the day of parturition (day 0), 20 Brown Swiss cows were assigned to either a control untreated group (control; n=6) or one of two groups in which cows were treated with Flunixin Meglumine (FM; Banamine, Schering Corp., Kenilworth, NJ), a prostaglandin synthetase inhibitor. Cows treated with FM were infused continuously for the first 10 days postpartum with either physiological saline (FM + Saline group; n=7) or prostaglandin $F_{2\alpha}$-tromethamine ($PGF_{2\alpha}$ Tham salt; The Upjohn Company, Kalamazoo, MI; FM + $PGF_{2\alpha}$ group, n=7). Protocol for inhibition of prostaglandin synthesis and $PGF_{2\alpha}$ replacement therapy is described below. During the entire experimental period (days 1 to 60 postpartum), cows were neither suckled or milked.

Within 12 h after parturition, at which time fetal membranes had been expelled, surgery was performed on cows assigned to the FM + saline and FM + $PGF_{2\alpha}$ groups. Surgery consisted of catheterization of the descending aorta via the dorsal costoabdominal artery. The costoabdominal artery is located on the caudal side of the last (13th) rib and is the most caudal of 13 paired arteries arising directly from the dorsal face of the aorta. During surgery, the cow was restrained in a chute with headgate and removable side bars. The area over the most caudal rib was shaved to prepare a surgical field 20 cm x 30 cm and the skin was scrubbed with germidicidal soap (Medadine, Med-Tech Inc., Elwood, KS). Local anesthesia was obtained with a point nerve block of the nerve accompanying the costoabdominal artery. This was accomplished by injecting approximately 30 ml of 2% lidocaine hydrochloride (Med-Tech Inc., Elwood, KS) into the epaxial musculature and then over the caudal border of the last rib. An additional 10 ml of 2% lidocaine hydrochloride was infiltrated subcutaneously cranial and dorsal to the area of incision and the surgical field was scrubbed thoroughly with germidicidal soap (Medadine, Med-Tech Inc., Elwood, KS).

A 10 cm straight skin incision was made over and parallel to the caudal border of the last rib with the dorsal end of the incision 2 cm from the epaxial musculature. The superficial lumbar facia and the serratus dorsalis caudalis muscle were carefully blunt dissected and the pulsing costoabdominal artery was located by palpation. A segment of the costoabdominal artery was exposed and a sterile polyvinyl catheter (0.86 mm id x 1.52 mm od; V-6, Bolab Inc., Derry, NH) was inserted. Catheter was advanced 35 to 40 cm into the descending aorta so that the end of the catheter was placed approximately 10 to 12 cm frontal to the iliac arteries. Therefore, experimental infusions via this catheter allowed equal perfusion of both uterine horns. The catheter was exteriorized through the incision which was closed with silk suture (Ethicon Inc., Somerville, NJ). The infusion catheter was wrapped in gauze and stored in a reinforced pouch placed over the incision site and sutured to the skin. Immediately after surgery, cows were given a single intramuscular injection of ampicillin (Polyflex, Bristol Laboratory, Syracuse, NY).

Replacement Therapy

Following surgery, cows in FM + saline and FM + $PGF_{2\alpha}$ groups were moved into an experimental barn where replacement therapy was conducted over the first 10 days postpartum. Endogenous production of prostaglandins was inhibited in the FM + saline and FM + $PGF_{2\alpha}$ groups by I.M. injections of FM, a prostaglandin synthetase inhibitor, administered at a dose of 1 g (20 ml) per injection. First injection of FM was given approximately 2 h after surgery. Subsequently, and until day 6 postpartum, FM was injected twice daily at 0800 h and 1700 h.

A stock solution of $PGF_{2\alpha}$ tham salt (7 mg/ml) was prepared by dissolving 350 mg $PGF_{2\alpha}$ tham salt into 50 ml of sterile 0.9% physiological saline. The stock solution was stored at 4 C. During the infusion period, fresh experimental solutions of $PGF_{2\alpha}$ tham salt were prepared daily by diluting either 5.2 ml (final concentration = 0.728 mg/ml) or 10.4 ml (final concentration = 1.456 mg/ml) of $PGF_{2\alpha}$ tham salt stock solution to a volume of 50 ml with sterile 0.9% physiological saline.

Continuous infusion of $PGF_{2\alpha}$ tham salt into the descending aorta was started on the morning of day 1 postpartum (FM + $PGF_{2\alpha}$ group), immediately after the second injection of FM had been administered. The free end of the catheter was connected to a 50 ml plastic syringe containing infusion solution of $PGF_{2\alpha}$ tham salt. The syringe was fixed to an infusion pump (Model 2201, Harvard

Apparatus, South Natick, MA) set to deliver 0.0323 ml per minute. Cows in the FM + $PGF_{2\alpha}$ were infused continuously (24 h/day) with a solution of $PGF_{2\alpha}$ tham salt from days 1 to 10 at a physiological dose of 33.86 mg/day [delivery rate = ($PGF_{2\alpha}$ tham salt, 0.728 mg/ml)(0.0323 ml/min)(60 min/h)(24 h/day)] from days 1 to 3 and from days 7 to 10 postpartum. Daily infusion dose of $PGF_{2\alpha}$ tham salt was increased to 67.72 mg/day [delivery rate = ($PGF_{2\alpha}$ tham salt, 1.456 mg/ml)(0.0323 ml/min)(60 min/h)(24 h/day)] from days 4 to 6 postpartum. Since 1.34 mg of $PGF_{2\alpha}$ tham salt is equivalent to 1.00 mg of free $PGF_{2\alpha}$, daily doses of 33.86 and 67.72 mg of $PGF_{2\alpha}$ tham salt were equivalent to doses of 25.26 and 50.53 mg of free $PGF_{2\alpha}$, respectively. These daily doses of $PGF_{2\alpha}$ were based upon estimation of F series prostaglandin production rates during the early postpartum period as determined in Chapter IV and considered to be in a physiological range. Cows in the FM + saline group were infused continuously with sterile 0.9% physiological saline vehicle at a rate of 46.5 ml/day [delivery rate = (0.0323 ml/min)(60 min/h)(24 h/day)] from days 1 to 10 postpartum. Cows in the control group did not have surgery or received FM or $PGF_{2\alpha}$ tham salt and were maintained in a pasture lot adjacent to the barn. After completion of the infusion period on day 11, cows in the FM + saline and FM + $PGF_{2\alpha}$ groups were kept in a pasture lot along with control untreated animals. During the experimental period, cows were fed hay and silage and given water ad libidum.

Data Collection

Blood samples (10 ml) were collected via jugular venipuncture into cold heparinized (100 i.u./tube) vacutainer tubes. In the FM +

saline and FM + $PGF_{2\alpha}$ groups, cows were bled twice daily from days 1 to 10, once daily from days 11 to 15 and thrice weekly (Monday, Wednesday, and Friday) from days 16 to 60 postpartum. During the period when FM was administered twice a day in the FM + saline and FM + $PGF_{2\alpha}$ groups (Days 0 to 5), blood samples were collected immediately prior to injection of FM. Cows in the control group were bled once daily from days 0 to 15 postpartum and thrice weekly (Monday, Wednesday, and Friday) from days 16 to 60. Upon collection, blood samples were placed immediately into an ice bath; within 30 min after collection, samples were centrifuged at 5000 x g for 15 min at 4 C and plasma stored at -20 C until sample analyses.

Starting on day 6 postpartum, the reproductive tract of each cow was palpated rectally thrice weekly, until day 60 postpartum. Blood sampling was performed immediately prior to palpation. Recorded measurements on the reproductive organs by palpation were: cervical diameter (mm), diameter of each uterine horn (mm) at the external bifurcation, uterine location (0, the uterus was completely forward of the pelvic floor; 50, approximately 50% of the uterus was in the pelvic canal; 100, all of the uterus was in the pelvic canal), ovarian volume (cm^3; length x width x thickness of each ovary) and presence or absence of corpus luteum (CL) and/or follicle (greater than 5 mm) on each ovary. The gravid uterine horn was determined by rectal palpation of uterine arteries at approximately 200 days of gestation. This first assessment of the gravid uterine horn was confirmed later by a postpartum rectal palpation of the reproductive tract within 24 h of parturition. With the exception of cervical diameter and uterine location, all measurements made on reproductive organs at the time of rectal palpation were recorded in relation to the previously gravid uterine horn.

Assay Procedures

Concentrations of 15-keto-13,14-dihydro-$PGF_{2\alpha}$ (PGFM) were measured by radioimmunoassay in plasma samples collected from days 1 to 15 postpartum to assess replacement therapy. See Chapter III for description of the procedure and validation of the assay. The antiserum (gift from Dr. K. Kirton, the Upjohn Company, Kalamazoo, MI) was prepared in a goat against 15-keto-13,14-dihydro-$PGF_{2\alpha}$ conjugated at the C_1 carbon to serum albumin by the carbodiimide method (Cornette et al., 1974). Unextracted plasma samples were assayed, and separation of free and bound PGFM was done by protein precipitation with polyethylene glycol-6000. Twenty-six repeated measurements of a reference plasma sample collected from an early postpartum cow in seven assays gave intra- and interassay coefficient of variation of 11.7% and 3.16%, respectively.

Plasma progesterone (P_4) concentrations were measured by radioimmunoassay in samples collected from days 1 to 60 postpartum. A description and validation of the assay procedure have been reported previously (Knight et al., 1977; Eley et al., 1981a). The antiserum (gift from Dr. J.L. Fleeger, Texas A&M University) was prepared in rabbit against 11_{α} -hydroxy-progesterone hemisuccinate conjugated to bovine serum albumin. Plasma samples (100 and 200 ul) were extracted with a mixture (2 ml) of benzene:hexane (1:2) as described by Louis et al. (1973). Recovery of tritiated progesterone after extraction averaged 87.3%. Accuracy of the assay procedure was determined by measuring known quantities of exogenous progesterone added to ovariectomized cow plasma to achieve final concentration of 125, 250, 500, 1250 and 2500 pg/ml. Recovery of added (X) versus measured ($\hat{Y}$) progesterone concentration was described by linear regression ($\hat{Y}$ = 249.71 ± 1.03X; $R^2 = 0.95$). The 249.71 pg/ml intercept, estimated by linear regression, is in close agreement with concentrations of 262.5 ± 51.3 pg/ml determined in ovariectomized cow plasma alone. All samples were run in two large assays. Intra- and interassay coefficients of variation of 30 repeated measurements of a reference plasma sample were 10.1 and 0.2%, respectively.

Plasma concentrations of LH were measured by a modification of the procedure described by Niswender et al. (1969). Experimental plasma samples (200 and 300 ul) were pipetted into borosilicate tubes. Volume was increased to 500 ul with appropriate volume of 0.05 M phosphate buffer pH 7.4 containing 1% (W/V) bovine serum albumin (1% BSA-PB). Standard curves were replicate in triplicate utilizing standard LH (USDA-bLH-B-5; gift from USDA Animal Hormone and National Hormone and Pituitary Program) at concentrations of 0.06, 0.12, 0.25, 0.50, 1.00, 2.50 and 5.00 ng per tube in 1% BSA-PB and bringing them to a volume of 500 ul using the same buffer. Two hundred ul of rabbit anti-bovine LH (AB-B-225; gift from Dr. G.D. Niswender, Colorado State University), diluted to 1:140,000 with normal rabbit serum [NRS; 1:400 dilution in EDTA buffer (18.6 g of EDTA in 1 l of phosphate buffer-saline (0.8% NaCl), pH 7.0; (NRS-EDTA-PBS-pH7.0)] was added to each tube except total count (TC) and nonspecific binding (NSB) tubes. All tubes were incubated for 24 h at 4 C, and then 50,000 cpm of ^{125}I-LH per 100 ul was added to each tube and incubated for 24 h at 4 C. The second antibody (serum antirabbit-guinea pig gamma globulin-SARGPGG; gift from Dr. J.H. Britt, North Carolina State University), diluted 1:30 in NRS-EDTA-PBS-pH 7.0, was added to a 6% polyethylene glycol (PEG-6000) solution in 0.05 M phosphate buffer, pH 7.4 at a volume ratio of 20 ml of second antibody solution to 50 ml of 6% PEG-6000 solution. The second antibody PEG-6000 solution (700 ul) was added to each tube except TC. After mixing by agitation, tubes were incubated for 15 min at room temperature and centrifuged for 30 min at 4 C and 3000 x g. The supernatant was decanted, tubes inverted and pellets allowed to dry for approximately 30 min. Tubes with pellets were counted in a gamma counter for 30 seconds. Different volumes (25, 50, 100, 200 and 200 ul) of a plasma known to have a high concentration of LH (22 ng per ml) were assayed. The linear regression (R^2 = .98) for the logit plot of the displacement curve ($\hat{Y}$ = 9.20 - 2.39X; $\hat{Y}$ = ln of B/F, X = $\log_{10}$ of assay volume) was parallel to the displacement curve for the standard curve ($\hat{Y}$ = 11.70 - 2.17X where $\hat{Y}$ = ln B/F, X = $\log_{10}$ of standard LH concentration). Addition of .25, .5, 1, 2, 4, 6, 8, 10 and 12 ng of USDA-bLH-B-5 to 1 ml of cow plasma gave a linear recovery of LH ($\hat{Y}$ = .26 + 1.03X where $\hat{Y}$ = ng per ml of hormone recovered and X = ng per ml of hormone added, R^2 = .99). The intercept value closely approximated the LH concentration in the cow plasma, 0.3 ng per ml). The intra- and interassay coefficients of variation were 11.96% and 17.09% for a plasma sample from a ovariectomized (OVX) cow ($\overline{X}$ = 0.76 ng/ml) and 6.89% and 9.32% for a sample of OVX plasma containing 2 ng of exogenous LH per ml ($\overline{X}$ = 2.77 ng/ml).

Estradiol-17_β concentrations were determined by radioimmunoassay in plasma samples collected from days 1 to 60 postpartum. Plasma samples (2 or 3 ml) were extracted twice with three volumes of freshly opened diethyl ether (Fisher Company, Orlando, FL). Solvent phases, containing estradiol-17_β were pooled, evaporated to dryness and assayed for estradiol-17_β following modification of the method described by Lindberg et al. (1974).

A stock solution of authentic estradiol-17_β was prepared by dissolving 1 mg of 1,3,5,(10)estratriene-3,17_β-diol (Steraloid, Wilton, NH) in 500 ml benzene (stock solution = 10 ug/ml). A portion of the stock solution of authentic estradiol-17_β was further rediluted to 1 ug/ml with benzene and used for preparation of estradiol-17_β standard solutions. Appropriate volumes of diluted estradiol-17_β stock solution (1 ug/ml) were transferred to glass vials to achieve desired standard concentrations in 5 ml of PBSga. Prior to addition of 5 ml of PBSga to each glass vial, diluted estradiol-17_β stock solution (1 ug/ml) was evaporated to dryness under a stream of air. Standard solutions of estradiol-17_β in PGSga had final concentrations of 0, 6.25, 10, 12.50, 20, 25, 30, 40, 50 and 80 pg/100 ul.

Diethyl ether (3 ml) was evaporated in assay tubes used for estradiol-17_β standard curves to simulate conditions of extraction for experimental samples. In each assay, estradiol-17_β standard curves were replicated in duplicate and were prepared by pipetting 100 ul of each standard solution into assay tubes. Dried extracts of experimental plasma samples were solubilized in 100 ul PBSga. Antiserum (gift from Dr. G.H. Stabenfeldt, University of Davis, CA) was prepared in sheep against estradiol-17_β-6-(O-carboxymethyl)-oxime conjugated to bovine serum albumin (Lindberg et al., 1974) and added to each tube at a dilution of 1:80000 in 100 ul PBSga. A 100 ul aliquot of approximately

22,000 dpm of (6,7-^{3}H) estradiol-17$_\beta$ (53 Ci/mmole; New England Nuclear, Boston, MA) diluted in PBSga was added and assay tubes were incubated overnight at 4 C. Separation of free and bound hormone was performed by addition of 500 ul of a solution of dextran-coated charcoal (250 mg charcoal and 25 mg Dextran suspended in 100 ml PBSga). Samples were incubated in an ice bath for 15 minutes and centrifuged (4 C) for 15 min at 2000 g. Aliquot (500 ul) of supernatant was transferred into a scintillation vial and scintillation cocktail (20% triton-toluene; 2.5 ml) added.

Double extraction of estradiol-17$_\beta$ from cow plasma with diethyl ether was accompanied occasionally by extraction of colored pigments which caused a variable color quench. For each sample, correction for quench was made by first determining counting efficiency (E) using the channel ratio (A/B) provided with each determination of counts per minute (cpm) by the scintillation counter (Delta 300; Searle Analytic Inc., Des Plaines, IL). Determination of a quench curve using appropriate tritiated standards yielded an estimate of counting efficiency (E) described as: $E = 122.84 - 68.14\ (A/B) + 11.15\ (A/B)^2$. After determination of counting efficiency (E) for each experimental sample and for samples of the standard curves, counts per minute (cpm) were adjusted to an efficiency of 100% or dpm.

Accuracy of the assay procedure was determined by measuring, in two different assays, known quantities of exogenous estradiol-17$_\beta$ previously added to cow plasma. Masses of estradiol-17$_\beta$ added resulted in an increase of the original concentration of estradiol-17$_\beta$ in cow plasma by 5, 15 and 20 pg/ml. Samples were extracted and assayed in triplicate by the method previously described. Analysis of variance was performed on measured estradiol-17_β concentrations. The main effect of assay (A), concentration (C) and their interaction were tested using the General Linear Model procedure of Statistical Analysis System (SAS; Barr et al., 1979). Only the main effect of concentration was significant ($P < 0.01$). Recovery of added ($\overline{X}$) versus measured ($\hat{Y}$) estradiol concentration was described by linear regression ($\hat{Y} = 18.78 + 0.97X$; $R^2 = 0.88$). A minimum mass of 10 pg estradiol was required to displace tritiated estradiol-17_β from zero (96-98% binding; $P < 0.05$), thereby establishing sensitivity of the assay at 10 pg/tube. The 18.78 pg/ml intercept (estimated by linear regression) represented original concentration of estradiol measured in a plasma pool prior to addition of exogenous masses. Crossreactivity of antiserum to estradiol-17_β have been described in detail by Lindberg et al. (1974) and was 0.7%, 0.6% and 11.0% with estradiol-17_α, estriol and estrone, respectively. These also were confirmed in our laboratory. Recovery of tritiated estradiol-17_β added to cow plasma averaged 98% after double extraction with diethyl ether. Repeated measurement of a reference plasma sample in each assay resulted in intra- and inter-assay coefficient of variation of 12.9% and 10.8%, respectively. Although antibody used for determination of estradiol-17_β concentrations was highly specific (Lindberg et al., 1974), the term estradiol will be used in this chapter.

P_4 Rise and Data Transformation

Two different approaches were taken to evaluate plasma progesterone responses during the first 60 days after parturition. Initially, a rise in P_4 concentration was defined to occur when concentrations of P_4 increased above 1.0 ng/ml. A rise of P_4 was completed when concentrations of P_4 returned to less than 1.0 ng/ml. For each animal, within a group, the total number of P_4 rises was computed. In addition, using various programming options of the Statistical Analysis System (SAS; Barr et al., 1979), the following parameters defining each P_4 rise were calculated: timing (day), duration (days), mean P_4 concentration (pg/ml), magnitude (pg/ml) and summation of P_4 concentrations (pg/ml).

Analyses of P_4 rises dealt with large animal within group variation, and cows that did not show a P_4 rise were omitted from the analyses. Likewise, more conventional types of statistical analyses describing time trends of hormonal concentrations (Eley et al., 1981a) have limited efficiency since postpartum cows are not synchronized and show large variation in their cyclic activity during the first 60 days postpartum. Therefore, an attempt was made to transform the data so that among and within animal variation would be better controlled and that overall postpartum response of the animal would be integrated into one single function. The rationale for this transformation was that collectively, occurrence of a P_4 rise, the number of P_4 rises, and all individual parameters describing P_4 rises contributed to the overall postpartum response of the animal for the 60 day period. For each cow within a group, transformation was done by additively accumulating concentrations of P_4 over the 60 day period. The transformed value for a given day is therefore equivalent to the summation of P_4 concentrations up to that day. As illustrated in Figure 5.1, for a cow that showed two P_4 rises and initiated a third one, P_4 concentrations accumulated at a fast rate during

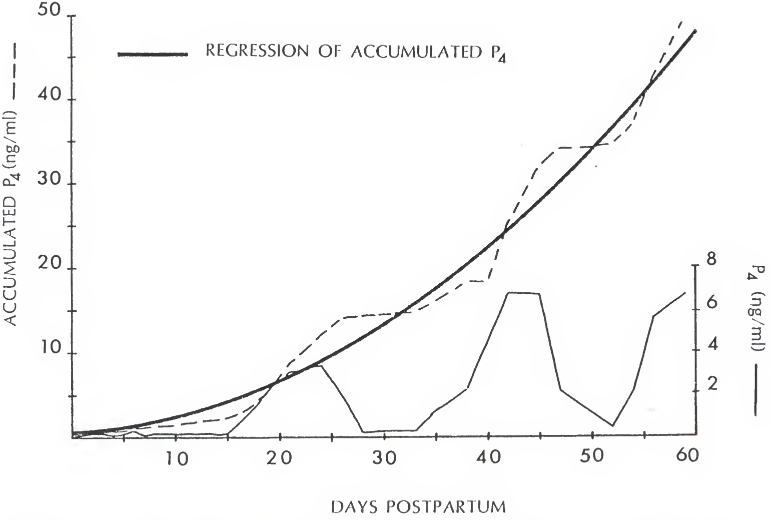


Figure 5.1 Illustration of original (-) and accumulated (---) values of progesterone concentrations (ng/ml) in a cow from days 1 to 60 postpartum; second order regression (——) describing the accumulated function.

luteal phases whereas the rate of accumulation was lower prior to or between luteal phases. In the case of cows that would show only one rise or that would not show any rise, the profile of P_4 accumulation would be much lower.

The same type of transformation was applied to determine the accumulation of estradiol concentrations and occurence of ovarian structures during the first 60 days postpartum. In the latter case, an arbitrary value of 1 was given when a follicle and/or a corpus luteum was present at the time of rectal palpation. A value of 0 was given when no significant structure could be palpated. Accumulation of 0 and 1 values provided an overall profile of ovarian activity during the 60 day period studied and complemented analyses of frequency of ovarian structures by the method of Chi-square.

Statistical Analyses

Each parameter defining a P_4 rise (e.g., timing, duration, mean P_4 concentration, magnitude and summation of P_4 concentration) were analyzed by least squares analysis of variance utilizing the General Linear Models procedures of the Statistical Analysis System (SAS; Barr et al., 1979). The mathematical model included sources of variation due to treatment, cow within treatment, rise number and the interaction of treatment with rise number.

Changes in variables (original and/or transformed values) measured in jugular blood and in gross morphology of the reproductive organs between treatment groups were analyzed by least squares analysis of variance according to the General Linear Models procedures of the Statistical Analysis System (SAS; Barr et al., 1979), and polynominal response curves were used to describe time trends.

The experimental design was in the nature of a split plot in which cows were nested within groups. Repeated measurements in each cow were taken over time which was considered a continuous independent variable. Group means (for original values) were tested against cow within group variation. In addition, tests of homogeneity of regressions were used to test differences in treatment response curves. When a test of homogeneity of regression was significant, orthogonal contrasts of treatment response curves were performed (control versus FM + saline and FM + $PGF_{2\alpha}$; FM + saline versus FM + $PGF_{2\alpha}$). Daily rates of accumulation of P_4 and estradiol (concentrations/day) were obtain by differentiation of polynomial equations generated to describe time trends for accumulation of these two hormones in each group.

A similar approach was used to compare palpable postpartum changes of reproductive organs according to the side on which measurement was made relative to the previously gravid uterine horn (e.g., uterine horn:previously gravid versus nongravid side; ovary:ipsilateral to previously gravid uterine horn versus contralateral side). Mathematical model included sources of variation due to treatment, cow within treatment, side, treatment by side and cow within treatment by side interaction. Day was considered a continuous independent variable and tests of homogeneity of regression used to detect differences in response curves between treatments, sides and the interaction of treatments and sides for the variables studied.

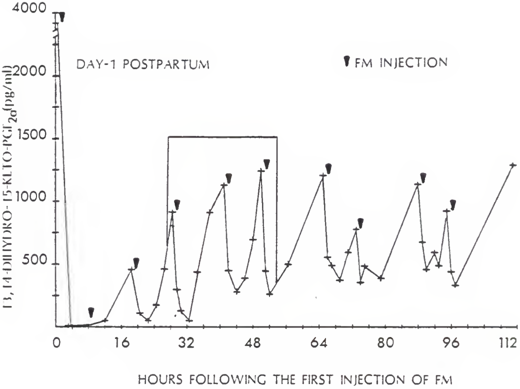
Baseline concentrations of estradiol and number and magnitude of spikes of estradiol were determined by a modification of the method of Christian et al. (1978). For each cow, linear and curvilinear time trends of estradiol concentrations were tested using polynomial regression analysis according to the General Linear Models procedure of the Statistical Analysis System (SAS; Barr et al., 1979). If a curvilinear time trend was detected, residual values (Christian et al., 1978), derived from proper polynomial equation, were used in subsequent analyses. A spike of estradiol was defined as a concentration value that was higher than the mean baseline concentration of the unskewed data set plus two standard deviations. Least squares analysis of variance utilizing the General Linear Models procedure of the Statistical Analysis System (SAS; Barr et al., 1979) was used to test differences between treatment in overall and baseline concentrations of estradiol as well as in number and magnitude of spikes.

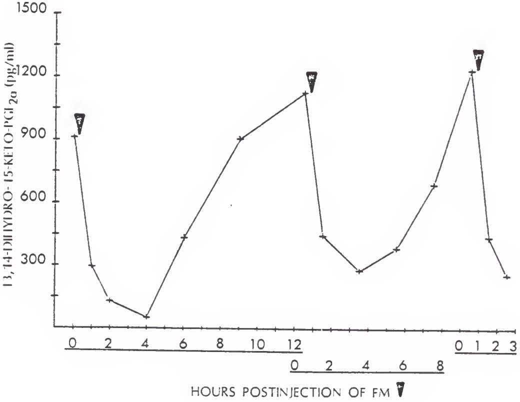
One cow assigned to the FM + $PGF_{2\alpha}$ group had twins and severe mastitis developed in one cow in the FM + saline group. Both animals were removed from the experiment.

Results

Efficiency of Flunixin Meglumine to inhibit synthesis of $PGF_{2\alpha}$ was evaluated in one postpartum Holstein cow (not included in the experiment). FM was injected twice daily according to the protocol described previously, and the cow was bled frequently via jugular venipuncture at approximately 2 h intervals for the first 5 days postpartum (i.e., 112 h following the first injection of FM; Figure 5.2). Following FM injections, peripheral concentrations of PGFM decreased and were minimal after 4 h. PGFM concentrations increased thereafter until the next injection of FM was given and reflected increased endogenous production of prostaglandins associated with metabolic clearance of FM by the animal. A comparable response of

Figure 5.2 Profile of inhibition of F series prostaglandins, as reflected by concentration of 15-keto-13-14-dihydro-PGF$_{2\alpha}$ (PGFM), after repeated i.m. injections of Flunixin Meglumine (FM) were given twice daily (0800 h and 1700 h) to a cow from day 1 to day 5 postpartum. Upper panel depicts the PGFM response after each FM injection for 112 h following the first injection of FM. Typical response of PGFM to an FM injection is enlarged in the lower panel.


4000
2000
1500
1000
500
13,14-DIHYDRO-15-KETO-PGF$_{2\alpha}$ (pg/ml)
DAY-1 POSTPARTUM
FM INJECTION
0
16
32
48
64
80
96
112
HOURS FOLLOWING THE FIRST INJECTION OF FM


1500
1200
900
600
300
13,14-DIHYDRO-15-KETO-PGF$_{2\alpha}$ (pg/ml)
0 2 4 6 8 10 12
0 2 4 6 8
0 1 2 3
HOURS POSTINJECTION OF FM

PGFM was repeated after each FM injection was given during the first 5 days postpartum.

In both the FM + saline and FM + $PGF_{2\alpha}$ groups, peripheral PGFM concentrations were higher ($P < 0.05$) in plasma samples collected prior to FM injection at 0800 h than in those collected at 1700 h. Since time intervals between samples collected prior to FM injection were different (i.e., time intervals were 15 h and 9 h for samples collected at 0800 h and 1700 h, respectively), metabolic clearance of FM was higher for samples collected at 0800 h (Figure 5.2) and therefore endogenous production of prostaglandin was higher. This trend was similar in FM + saline and FM + $PGF_{2\alpha}$. Therefore, values of PGFM concentrations for each animal within a day (2 samples) were averaged in subsequent analyses to evaluate daily concentrations.

Least squares means of plasma PGFM concentrations were 1058, 611, and 812 pg/ml (S.E.M. = ±203 pg/ml) in the FM + $PGF_{2\alpha}$, FM + saline and control groups respectively, and were not different between groups for the day 1 to 11 period ($P > 0.1$; Table 5.1). Profiles of daily changes in peripheral plasma PGFM concentrations during the first 11 days postpartum are depicted in Figure 5.3 and were different between groups ($P < 0.01$; Tables 5.2 and 5.3). In the control group, PGFM concentrations attained peak values of 1800 pg/ml on day 3 and decreased progressively thereafter. Although PGFM concentrations increased progressively during the period FM was injected in the FM + saline group (day 0 to 5), they were appreciably lower than in the control and the FM + $PGF_{2\alpha}$ groups and never exceeded 900 pg/ml during the first 11 days postpartum. However, in the FM + $PGF_{2\alpha}$ group, peak concentrations (1800 pg/ml) of peripheral PGFM were

TABLE 5.1. Least squares means of plasma concentrations of 15-keto,13,14-dihydro $PGF_{2\alpha}$ (PGFM; day 1 to 12 postpartum), progesterone (day 1 to 60 postpartum) and LH (days 1 to 60 postpartum) in three treatment groups of cows.

	Treatment Group[a]			
	FM + $PGF_{2\alpha}$	FM + saline	Control	S.E.M.[b]
PGFM (pg/ml)	1058	611	823	±203
Progesterone (pg/ml)	1106	846	1340	±217
LH (ng/ml)	0.70	0.71	0.88	±0.09

[a] No overall treatment effect ($P > 0.1$).

[b] S.E.M. = standard errors of the mean; they are approximate, conservative, and similar for each group.

TABLE 5.2. Least squares analyses of variance of postpartum responses in three treatment groups of Brown Swiss cows. Responses not recorded and those recorded in relation to the previously gravid uterine horn were analyzed according to Model 1 and Model 2, respectively.

Model 1

Source of	PGFM		LH		Accumulated Progesterone		Accumulated Estradiol		Cervix Diameter		Location of Uterus		
Variation	df	MS[a]	df	MS	df	MS[a]	df	MS[a]	df	MS	df	MS	Error Term
Treatment	2	2.17	2	1.76	2	0.97	2	3.80	2	38.98	2	42.64	Cow(treatment)
Cow(treatment)	15	45.19**	15	2.06**	15	0.99**	15	7.11**	15	300.93**	15	22.62**	Residual
Days[b]	3	79.76**	3	0.81*	2	34.89**	2	32.50**	4	5261.25**	3	802.00**	Residual
Residual	276	2.55	498	0.21	607	0.46	607	0.13	353	18.80	354	2.71	

Model 2

	Horn Diameter		Ovarian Volume		Ovarian Activity[c]		CL occurrence[c]		
	df	MS	f	MS	df	MS	df	MS[a]	Error Term
Treatment	2	0.93	2	235.33	2	319.50	2	2.25	Cow(treatment)
Cow(treatment)	15	283.46**	15	773.33**	15	233.73	15	8.91**	Residual
Side[d]	1	7333.00**	1	679.12	1	1993.40**	1	55.07**	Cow(treatment)xSide
Treatment x Side	2	35.98	2	317.58	2	104.40	2	0.98	Cow(treatment)xSide
Cow(treatment)xSide	15	42.76	15	343.47**	15	145.13**	15	5.39**	Residual
Days[e]	5	19000.00**	5	69.75*	2	4124.00**	2	102.46**	Residual
Residual	649	13.89	687	22.25	746	3.26	746	0.22	

[a] MS = MS x 10^5; MS = mean square.
[b] See Table 5.3 for tests of homogeneity of regression.
[c] Accumulated values.
[d] Side refers to measurements made on the previously gravid uterine horn and its ipsilateral ovary versus measurements made on the contralateral uterine horn and ovary.
[e] See Table 5.7 for tests of homogeneity of regression.
** $P < 0.01$; * $P < 0.05$.

TABLE 5.3. Tests of homogeneity of regression and orthogonal contrast of postpartum responses in three treatment groups of Brown Swiss cows.

Response	Order of regression	Error term		Treatment		Orthogonal contrast[c]	
		df[a]	MS[a]	df[b]	MS_D[b]	A	B
PGFM	2	276	255419	4	2333997**	**	+
LH	3	492	0.20	6	0.61**	**	NS
Accumulated progesterone	2	607	4639	4	64662**	**	**
Accumulated estradiol	2	607	13296	4	107681**	**	NS
Cervical diameter	4	353	18.8	8	37.2	NS	NS
Location of uterus (% in pelvic canal)	3	354	2.7	6	2.5	NS	NS

[a] Degrees of freedom (df) and mean square (MS) associated with the error term when individual curves were generated for each treatment.

[b] Degrees of freedom (df) and mean square (MS_D) associated with the difference in residual sums of squares between fitting one pooled curve versus an individual curve for each treatment.

[c] Orthogonal contrast: A = FM + saline, FM + $PGF_{2\alpha}$ versus Control
B = FM + saline versus FM + $PGF_{2\alpha}$.

** $P < 0.01$; + $P < 0.1$; NS = not significant.

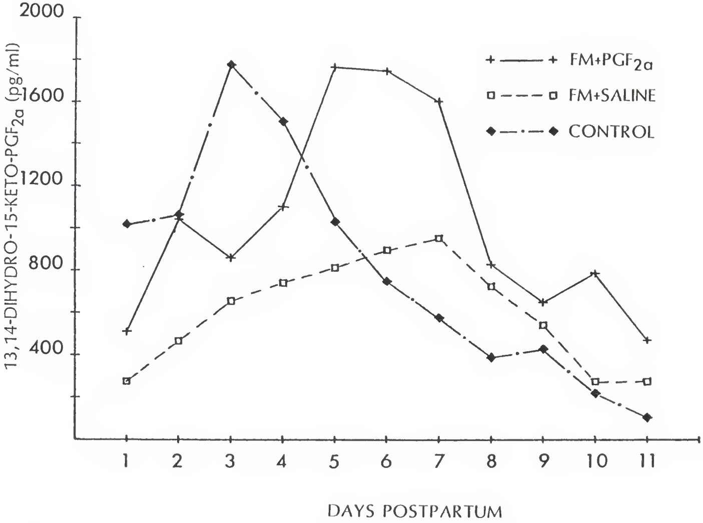


Figure 5.3 Profiles of least squares means for concentrations of 15-keto-13,14-dihydro-PGF$_{2\alpha}$ (PGFM) in cows injected from days 1 to 5 postpartum with a prostaglandin synthetase inhibitor (FM) and infused continuously from days 1 to 10 postpartum with PGF$_{2\alpha}$ (FM + PGF$_{2\alpha}$) or physiological saline (FM + saline) and in untreated cows (control).

comparable to those of the control group but were delayed by 2 days. Peaks at this time coincided with an increase of the daily infusion of $PGF_{2\alpha}$ to 50.53 mg. When 25.26 mg of $PGF_{2\alpha}$ was infused daily (day 1 to 3 and 7 to 10), peripheral PGFM concentrations in the FM + $PGF_{2\alpha}$ group ranged from approximately 800 to 1100 pg/ml. Although PGFM concentrations were appreciably lower in the FM + saline group, a temporal increase between day 1 and 7 was detected (Figure 5.3). Therefore, suppression rather than total inhibition of endogenous production of prostaglandins was achieved in the FM + saline group. Following replacement with $PGF_{2\alpha}$ in the FM + $PGF_{2\alpha}$ group, concentrations of PGFM measured in peripheral circulation indicated that rate of infusion of $PGF_{2\alpha}$ was within the physiological range.

Postpartum least squares means of progesterone concentrations during the first 60 days postpartum did not differ between groups ($P > 0.1$; Table 5.1) and were 1106, 846 and 1340 pg/ml (S.E.M. = ±217) in the FM + $PGF_{2\alpha}$, FM + saline and control groups, respectively. Least squares means of parameters describing the first and second postpartum P_4 rises among the 18 animals used in this experiment are presented in Table 5.4. Values of duration, mean P_4 concentration, magnitude and summation of P_4 concentrations of the first P_4 rise were 52.4%, 41.2%, 53.0% and 62.0% lower, respectively, ($P < 0.01$) than those of the second P_4 rises.

No statistical differences between treatment groups were detected for any of the parameters describing the first and second P_4 rise ($P > 0.1$; Table 5.5). However, for each of these parameters, tendencies were observed that contributed collectively to the overall accumulated progesterone responses that differed among groups. Five

TABLE 5.4. Least squares means (± S.E.M.) for parameters of the 1st and 2nd postpartum (day 1 to 60) P_4 rises (P_4 > 1 ng/ml) in cows: timing of rise, duration of rise, P_4 concentration (conc.) of rise, magnitude of rise and summation of P_4 under curve.

Rise number *	Timing (day)	Duration (days)	P_4 conc. (pg/ml)	Magnitude (pg/ml)	Summation (pg/ml)
1st rise	30.05±0.93	5.00±1.01	2489±552	2821±499	7447±2197
2nd rise	46.06±1.13	10.52±1.22	4234±522	6027±605	19674±2661

*1st rise responses are all significantly less ($P < 0.01$) than 2nd rise responses.

TABLE 5.5. Least squares means (± S.E.M.) for parameters of the first and second postpartum (day 1 to 60) P_4 rise ($P_4 > 1$ ng/ml) in three treatment groups of cows: timing of rise, duration of rise, P_4 concentration (conc.) of rise, magnitude of rise and summation of P_4 under curve.

	Frequency of cows showing a rise	Timing[b] (day)	Duration[b] (days)	P_4 conc.[b] (pg/ml)	Magnitude[b] (pg/ml)	Summation[b] P_4 (pg/ml)
1st rise						
FM + $PGF_{2\alpha}$	6/6	27.16±4.50	4.16±1.40	2397±562	2794± 533	6145±2346
FM + Saline	5/6	38.33±6.25	4.66±1.97	2409±573	2530±1261	6738±4807
Control	6/6	24.66±6.69	6.16±2.35	2662±520	3229± 923	9454±3834
2nd rise						
FM + $PGF_{2\alpha}$	6/6 (4/6)[a]	40.83±4.71	10.33±2.77	3884±430	5453± 849	18806±4930
FM + Saline	4/6 (4/4)	53.08±2.76	8.66±2.47	4133±618	5401±1221	16414±5383
Control	5/6 (4/5)	44.26±3.15	12.56±2.07	4684±519	7227±1023	23802±4504

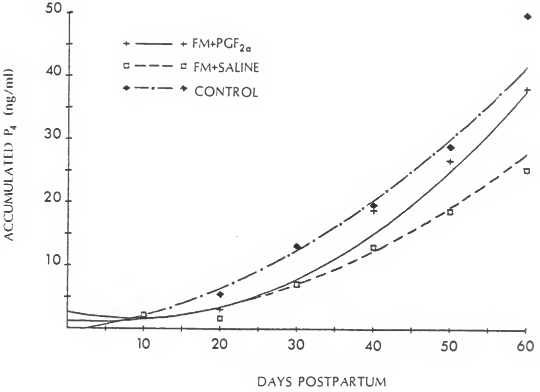
[a] Frequency of cows showing a complete second P_4 rise in parentheses.

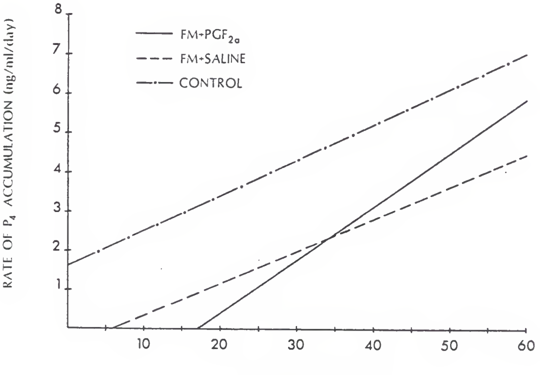
[b] No treatment effect ($P > 0.1$).

of six cows in the FM + saline group had a first P_4 rise that was delayed by an average of 11 and 14 days in comparison to the FM + $PGF_{2\alpha}$ and control groups, respectively. Likewise, four of six cows in the FM + saline group had a second P_4 rise which on the average, occurred at 53 days postpartum and was completed in all cases by day 60 postpartum (duration = 8.66 days). In the FM + $PGF_{2\alpha}$ and control groups, the second P_4 rise tended to occur earlier (40 and 44 days, respectively) than in the FM + saline group and lasted a minimum of 10 and 12 days, respectively. Values for mean progesterone concentrations, magnitude and summation of P_4 concentrations of the first and second rise were not different between groups ($P > 0.1$) although they tended to favor the control group. Accumulation of P_4 concentrations over the first 60 days postpartum for each cow within group resulted in the integration of each of these tendencies into one single response function. This approach provided a sensitive test of the overall P_4 response of postpartum cows between groups.

Profile of accumulated P_4 concentrations and rate of accumulation of progesterone concentrations during the first 60 days postpartum are depicted in Figure 5.4. Profile of accumulated P_4 concentrations was altered significantly by early postpartum modulation of prostaglandin production ($P < 0.01$; Table 5.3). Profiles of accumulated P_4 concentrations and the rate of accumulation of P_4 were higher ($P < 0.01$; Table 5.3) in the control group than in the FM + $PGF_{2\alpha}$ and FM + saline groups. Profiles of accumulated P_4 concentrations were similar in FM + saline and FM + $PGF_{2\alpha}$ groups during the first 25 days postpartum, but diverged thereafter when rate of accumulation of progesterone progressively increased in FM + $PGF_{2\alpha}$

Figure 5.4 Least squares regressions and least squares means (every tenth day) of accumulated progesterone concentrations (upper panel) and rate of accumulation of progesterone (lower panel) from days 1 to 60 postpartum in cows injected from days 1 to 5 postpartum with a prostaglandin synthetase inhibitor (FM) and infused continuously from days 1 to 10 postpartum with $PGF_{2\alpha}$ (FM + $PGF_{2\alpha}$) or physiological saline (FM + saline) and in untreated cows (control).


50
40
30
20
10
ACCUMULATED P_4 (ng/ml)
+ ——— + FM+$PGF_{2\alpha}$
□ – – – □ FM+SALINE
◆ —·— ◆ CONTROL
10
20
30
40
50
60
DAYS POSTPARTUM


8
7
6
5
4
3
2
1
RATE OF P_4 ACCUMULATION (ng/ml/day)
——— FM+$PGF_{2\alpha}$
– – – FM+SALINE
—·— CONTROL
10
20
30
40
50
60
DAYS POSTPARTUM

group to levels comparable to those of the control group ($P < 0.01$; Table 5.3).

Mean concentrations of LH from days 1 to 60 postpartum were 0.70, 0.71 and 0.80 ng/ml (S.E.M. = ± 0.09) in the FM + $PGF_{2\alpha}$, FM + saline and control group respectively and did not differ ($P > 0.1$; Tables 5.1 and 5.2). However, profiles of LH were not parallel between groups from days 1 to 30 postpartum ($P < 0.01$; Tables 5.2 and 5.3; Figure 5.5). Initially, from days 1 to 10 postpartum, concentrations of LH increased more rapidly in the control group and remained appreciably higher ($P < 0.01$; Tables 5.2 and 5.3) than in the FM + $PGF_{2\alpha}$ and FM + saline groups. During the same period, LH concentrations increased slowly and similarly in both the FM + $PGF_{2\alpha}$ and FM + saline groups ($P > 0.1$; Table 5.3).

There was no difference in either overall, baseline and spike concentrations of estradiol between treatment groups ($P > 0.1$; Table 5.6). For the three groups, mean overall, baseline and spike concentrations of estradiol were 10.4, 5.1 and 40.5 pg/ml, respectively. Likewise, frequency of spikes did not differ between groups ($\chi^2 = 2.34$; $P > 0.1$). However, after adjustment for variation between cows, residual variance was appreciably higher in the control group than in the FM + saline and FM + $PGF_{2\alpha}$ groups (4123 versus 550 and 303, respectively; Table 5.6). Variability of estradiol concentrations within cows of each treatment group included day to day and random variation which may be related to degree of follicular activity (growth and atresia) as previously suggested (Echternkamp and Hansel, 1973; Fernandes et al., 1978b; Stevenson and Britt, 1979).

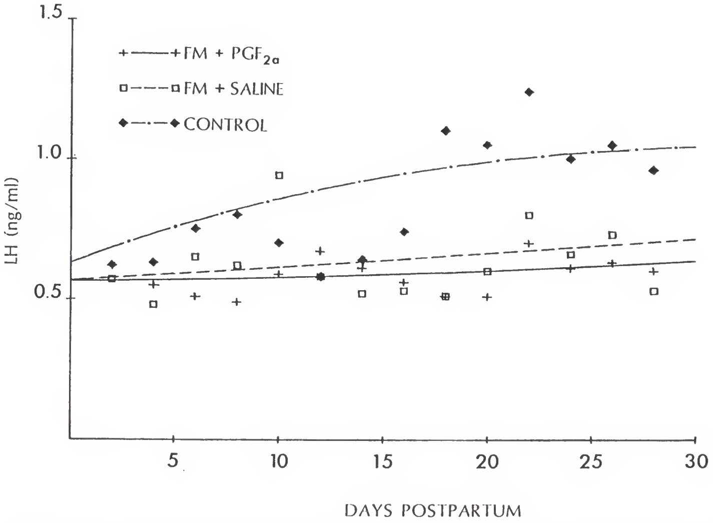


Figure 5.5 Least squares regressions and least squares means of LH concentrations from days 1 to 30 postpartum in cows injected from days 1 to 5 postpartum with a prostaglandin synthetase inhibitor (FM) and infused continuously from days 1 to 10 postpartum with $PGF_{2\alpha}$ (FM + $PGF_{2\alpha}$) or physiological saline (FM + saline) and in untreated cows (control).

TABLE 5.6. Least squares means (± S.E.M.) of plasma estradiol concentrations, total number of spikes and residual variance during the first 60 days postpartum in three treatment groups of cows.

	Least squares means[a]				
	Overall[b] pg/ml	Baseline[c] pg/ml	Spike[c] pg/ml	Number[a,c] of spikes	Residual[b] variance
FM + $PGF_{2\alpha}$	9.9±1.2	5.8±0.8	38.1±29.2	23	303
FM + saline	8.3±1.7	4.8±0.8	34.1±29.2	20	550
Control	13.1±4.7	4.7±0.8	49.2±29.2	30	4123

[a] No treatment effect ($P > 0.1$).

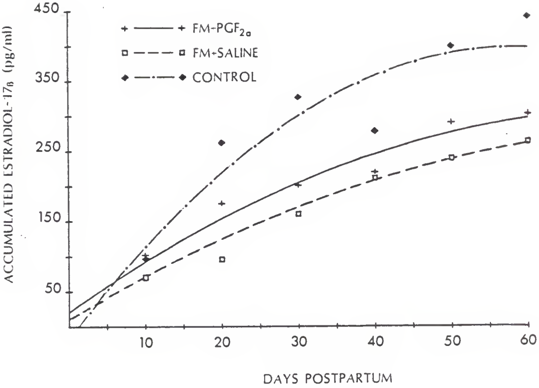
[b] Heterogeneity of variance ($P < 0.01$).

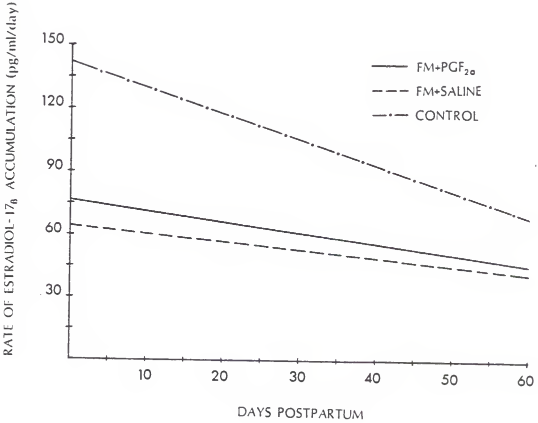
[c] Determined after modification of the method described by Christian et al. (1978).

Significant among group variability for estradiol concentrations was further reflected in the profile of accumulated and rate of accumulation of estradiol concentrations (Figure 5.6). Test of homogeneity of regression indicated that profiles of accumulated estradiol were not parallel between groups ($P < 0.01$; Table 5.3). During the first 25 days postpartum, estradiol concentrations accumulated at a faster rate in the control group and therefore, profile of accumulated estradiol was higher than in FM + saline and FM + $PGF_{2\alpha}$ groups; however, profiles of accumulated estradiol were not different between the FM + saline and FM + $PGF_{2\alpha}$ groups ($P > 0.1$; Table 5.3).

There were no differences in mean cervical diameters, location of uteri and uterine horn diameters between treatment groups ($P > 0.1$; Table 5.2). Likewise, postpartum changes in cervical diameter and in location of the uterus were not different between treatments ($P > 0.05$; Table 5.3; Figure 5.7). Cervical diameter decreased from mean values of 58 mm on day 6 to 32 mm on day 35 postpartum and remained constant thereafter. Percentage of uterus in the pelvic canal was low initially and increased rapidly thereafter. By day 30 postpartum, uteri had returned completely into the pelvic canal. Postpartum decreases in uterine horn diameter of previously gravid and nongravid uterine horns were similar among groups ($P > 0.05$; Table 5.7; Figure 5.8). However, reduction in uterine horn diameter was more rapid for the previously gravid than nongravid uterine horns ($P < 0.01$; Table 5.7; Figure 5.8). Initially (day 6), mean diameters of the previously gravid and nongravid uterine horns were 90 and 50 mm respectively; by

Figure 5.6 Least squares regressions and least squares means (every tenth day) of accumulated estradiol concentrations (upper panel) and rate of accumulation of estradiol (lower panel) from days 1 to 60 postpartum in cows injected from days 1 to 5 postpartum with a prostaglandin synthetase inhibitor (FM) and infused continuously from days 1 to 10 postpartum with PGF$_{2\alpha}$ (FM + PGF$_{2\alpha}$) or physiological saline (FM + saline) and in untreated cows (control).


450
350
250
150
50
ACCUMULATED ESTRADIOL-17_β (pg/ml)
FM-PGF$_{2\alpha}$
FM+SALINE
CONTROL
10
20
30
40
50
60
DAYS POSTPARTUM


150
120
90
60
30
RATE OF ESTRADIOL-17_β ACCUMULATION (pg/ml/day)
FM+PGF$_{2\alpha}$
FM+SALINE
CONTROL
10
20
30
40
50
60
DAYS POSTPARTUM

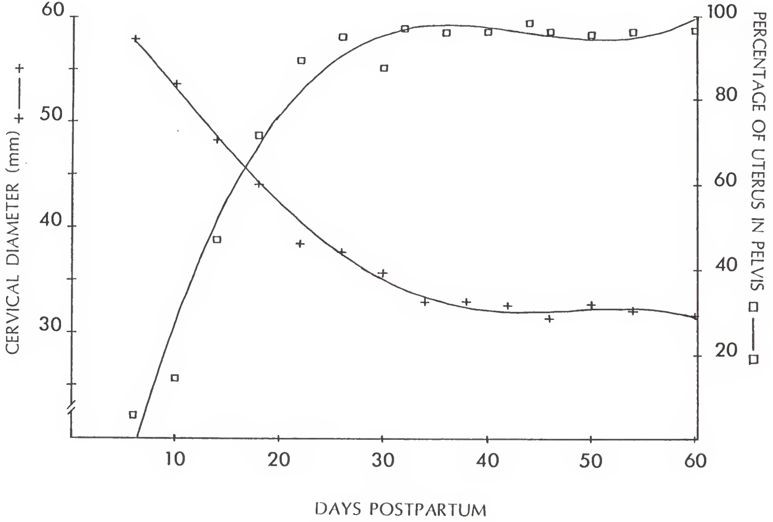


Figure 5.7 Pooled least squares regression and least squares means (every fourth day) of cervical diameters and of locations of uterus from days 6 to 60 postpartum in cows injected from days 1 to 5 postpartum with a prostaglandin synthetase inhibitor (FM) and infused continuously from days 1 to 10 postpartum with $PGF_{2\alpha}$ (FM + $PGF_{2\alpha}$) or physiological saline (FM + saline) and in untreated cows (control).

TABLE 5.7. Tests of homogeneity of regression for changes in postpartum responses measured ipsilateral and contralateral (Side) to the previously gravid uterine horn during the first 60 days postpartum in three treatment groups of Brown Swiss cows.

Response	Order of Regression	Error term		Treatment		Side		Treatment x Side	
		df[a]	MS[a]	df[b]	MS_D[b]	df[b]	MS_D[b]	df[b]	MS_D[b]
Uterine horn diameter	5	649	13.89	10	26.93	5	636.40**	10	5.00
Ovarian volume	5	687	22.25	10	40.28*	5	122.50**	10	40.85*
Ovarian activity	2	746	3.26	4	38.60**	2	229.40**	4	19.07**
CL occurrence	2	746	22054	4	70036*	2	936378**	4	101105**

[a] Degrees of freedom (df) and mean square (MS) associated with the error term when individual curves are generated for each treatment and each side.

[b] Degrees of freedom (df) and mean square (MS_D) associated with the difference in residual sums of squares between fitting for each measurements one pooled curve versus an individual curve for each treatment, an individual curve for each side (ipsilateral or contralateral) or an individual curve for measurements made on each side within each treatment.

** $P < 0.01$; * $P < 0.05$.

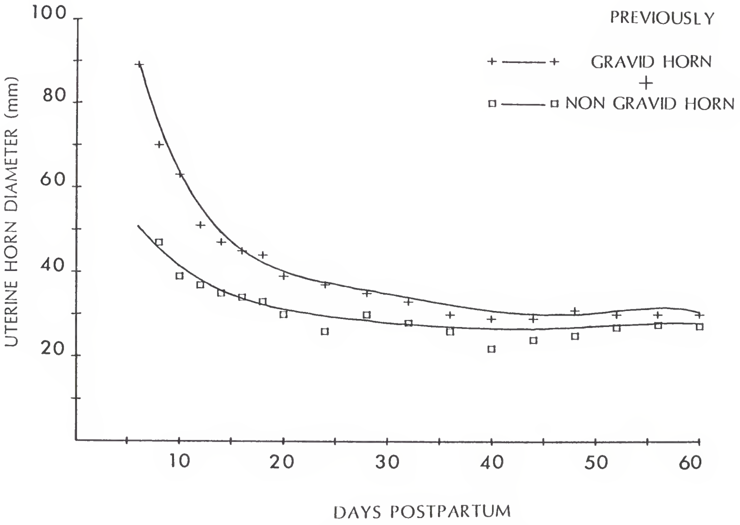


Figure 5.8 Pooled least squares regressions and least squares means (every fourth day) of previously gravid and nongravid uterine horn diameter from days 6 to 60 postpartum in cows injected from days 1 to 5 postpartum with a prostaglandin synthetase inhibitor (FM) and infused continuously from days 1 to 10 postpartum with $PGF_{2\alpha}$ (FM + $PGF_{2\alpha}$) or physiological saline (FM + saline) and in untreated cows (control).

day 35, reduction in uterine horn diameter was completed and size of both uterine horns was similar.

Initial changes in ovarian volume of postpartum ovaries ipsilateral to the previously gravid uterine horn are associated with regression of the CL of pregnancy. Test of homogeneity of regression indicated that changes in ovarian volume on the ipsilateral side were not parallel between groups ($P < 0.05$; Table 5.7; Figure 5.9). Initially, from days 6 to 15 postpartum, the ovarian volume of ovaries ipsilateral to the previously gravid horn was approximately 30% larger and decreased less rapidly in the FM + saline group than in the control or FM + $PGF_{2\alpha}$ groups. In contrast, ovarian volumes of contralateral ovaries (not bearing the CL of pregnancy) were not reduced early postpartum and changes were similar between groups.

Number and percent of ovaries, ipsilateral and contralateral to the previously gravid uterine horn, without significant palpable structures (inactive ovary) and with a palpable CL and/or follicle (active ovary) are presented in Table 5.8. Percentage of active ovaries was higher significantly ($\chi^2 = 41.09$; $P < 0.01$) for ovaries contralateral ($\overline{X} = 59.6\%$) than ipsilateral ($\overline{X} = 37.06\%$) to the previously gravid uterine horn. However, there was a significant interaction ($\chi^2 = 57.39$; $P < 0.01$) of treatment and location of the ovary, in relation to the previously gravid uterine horn, on ovarian activity. As compared to the untreated control group, administration of FM reduced the percent of active ovaries contralateral to the previously gravid uterine horn by 11.2 and 18.2 percentage points in the FM + saline and FM + $PGF_{2\alpha}$ groups, respectively. Likewise, percent of active ovary ipsilateral to the previously gravid uterine

Figure 5.9 Least squares regressions and least squares means (every fourth day) of ovarian volume of ovaries ipsilateral to the previously gravid uterine horn from days 6 to 24 postpartum in cows injected from days 1 to 5 postpartum with a prostaglandin synthetase inhibitor (FM) and infused continuously from days 1 to 10 postpartum with $PGF_{2\alpha}$ (FM + $PGF_{2\alpha}$) or physiological saline (FM + saline) and in untreated cows (control).

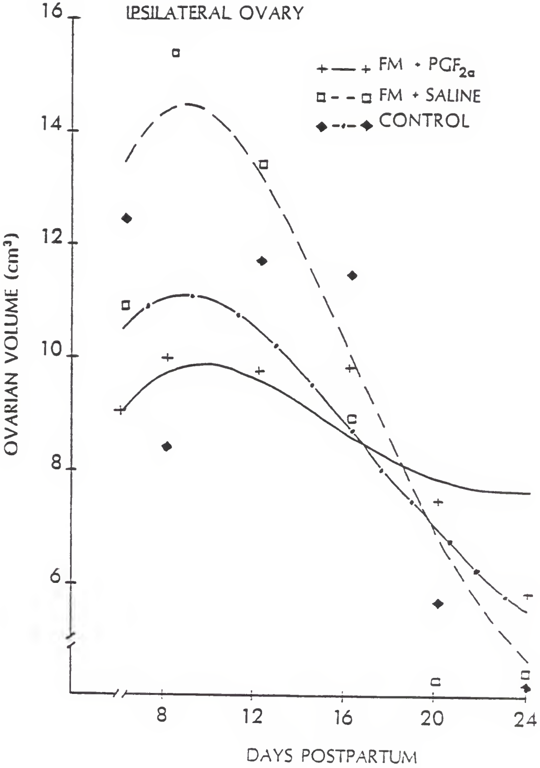

IPSILATERAL OVARY
+—+ FM + $PGF_{2\alpha}$
□- -□ FM + SALINE
◆-·-◆ CONTROL
OVARIAN VOLUME (cm^3)
16
14
12
10
8
6
8
12
16
20
24
DAYS POSTPARTUM

TABLE 5.8. Number and percent (%) of inactive and active ovaries ipsilateral (Ip) and contralateral (Con) to the previously gravid uterine horn in three treatment groups of postpartum cows (day 6 to 60).

	Treatment Group					
	FM + $PGF_{2\alpha}$		FM + Saline		Control	
	Ip	Con	Ip	Con	Ip	Con
Inactive[a]	69	62	88	53	81	42
Active[b]	56	66	34	75	51	97
Total	125	128	122	128	132	139
% Active**	44.8	51.5	27.8	58.5	38.6	69.7

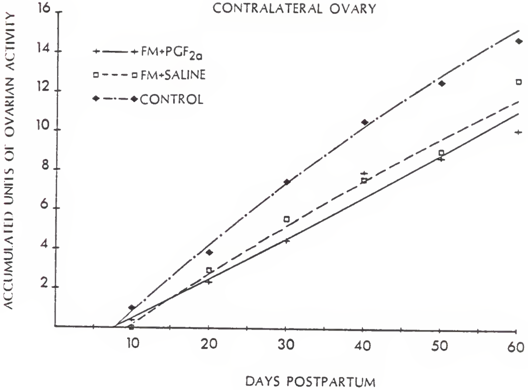
[a] Inactive = no significant palpable structure.
[b] Active = palpable CL and/or follicle.
** Treatment x side interaction ($P < 0.01$).

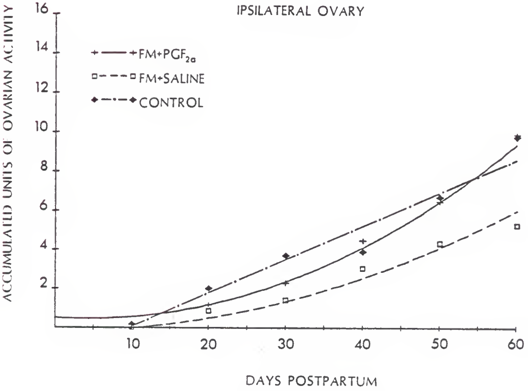
horn was 10.8% lower in the FM + saline group than in the control group. However, replacement with $PGF_{2\alpha}$ (FM + $PGF_{2\alpha}$) enhanced percent of active ovary on the ipsilateral side to level that was comparable to that of the control group (44.8 versus 38.6%, respectively).

A second approach to evaluate the interaction between treatment and side on which measurement was made to assess ovarian activity throughout the 60 day postpartum period. At each palpation, values of 0 and 1 were given to inactive and active ovaries, respectively. Units of ovarian activity (0 and 1) were accumulated over the first 60 days postpartum for ovaries contralateral and ipsilateral to the previously gravid uterine horn in the three treatment groups (Figure 5.10). Profile of accumulation of units of ovarian activity was higher for ovaries contralateral than ipsilateral to the previously gravid uterine horn and was different between treatments ($P < 0.01$; Table 5.7). However, there was a significant interaction of these two main effects ($P < 0.01$; Table 5.7). Units of ovarian activity for ovaries contralateral to the previously gravid uterine horn accumulated less rapidly in the FM + saline and FM + $PGF_{2\alpha}$ groups than in the control group. Treatment with FM reduced (FM + saline) the rate of accumulation of units of ovarian activity on ovaries ipsilateral to the previously gravid uterine horn but replacement with $PGF_{2\alpha}$ (FM + $PGF_{2\alpha}$) progressively increased the rate of accumulation of ovarian activity units to levels comparable to those of the control group.

Contribution of the presence of a CL to postpartum ovarian activity was examined by a similar approach. At each palpation,

Figure 5.10 Least squares regressions and least squares means (every tenth day) of accumulated units of ovarian activity from days 6 to 60 postpartum measured on ovaries contralateral (upper panel) and ipsilateral (lower panel) to the previously gravid uterine horn in cows injected from days 1 to 5 postpartum with a prostaglandin synthetase inhibitor (FM) and infused continuously from days 1 to 10 postpartum with $PGF_{2\alpha}$ (FM + $PGF_{2\alpha}$) or physiological saline (FM + saline) and in untreated cows (control).

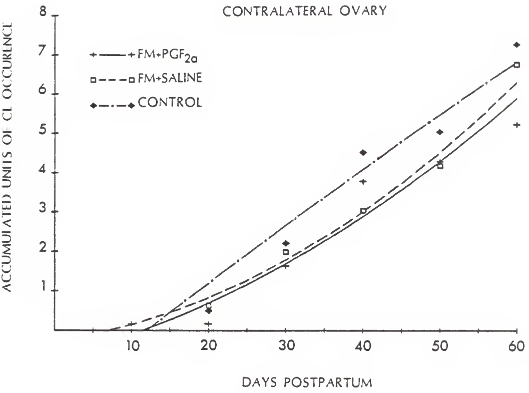

CONTRALATERAL OVARY
FM+PGF$_{2\alpha}$
FM+SALINE
CONTROL
ACCUMULATED UNITS OF OVARIAN ACTIVITY
16
14
12
10
8
6
4
2
10
20
30
40
50
60
DAYS POSTPARTUM


IPSILATERAL OVARY
FM+PGF$_{2\alpha}$
FM+SALINE
CONTROL
ACCUMULATED UNITS OF OVARIAN ACTIVITY
16
14
12
10
8
6
4
2
10
20
30
40
50
60
DAYS POSTPARTUM

values of 0 and 1 were given to ovaries without and with a palpable CL, respectively. Units of CL occurrence (0 and 1 values) were accumulated over the first 60 days postpartum for ovaries contralateral and ipsilateral to the previously gravid uterine horn in the three treatment groups (Figure 5.11). Profile of accumulated units of CL occurrence was higher for ovaries contralateral than ipsilateral to the previously gravid uterine horn ($P < 0.01$; Table 5.7), but there was a treatment by side interaction over the 60 day period studied ($P < 0.01$; Table 5.7). Groups of cows that received FM had a lower profile of accumulated units of CL occurrence than the control group on both the contralateral (FM + $PGF_{2\alpha}$; FM + saline) and ipsilateral ovaries (FM + saline). However, after replacement with $PGF_{2\alpha}$ (FM + $PGF_{2\alpha}$), there was a selective increase in the profile of accumulated units of CL occurrence on ovaries ipsilateral to the previously gravid uterine horn. This positive and selective effect, observed at the ovarian level, due to replacement therapy with an infusion of $PGF_{2\alpha}$ was reflected by a higher profile of accumulated progesterone concentrations in the FM + $PGF_{2\alpha}$ than in the FM + saline group after 30 days postpartum (Figure 5.4).

On a within cow basis, plasma PGFM concentrations were correlated positively (Table 5.9) with cervical ($P < 0.01$) and mean uterine horn diameter ($P < 0.1$) and negatively with location of the uterus ($P < 0.01$) in the pelvic canal. While estradiol concentrations were not correlated significantly ($P > 0.1$) with any of the responses studied, progesterone was correlated negatively ($P < 0.01$) with cervical and uterine horn diameter and positively ($P < 0.01$) with location of the uterus and ovarian volume. Interrelationships of the decrease in cervical and

Figure 5.11 Least squares regressions and least squares means (every tenth day) of accumulated units of CL occurrence from days 6 to 60 postpartum measured on ovaries contralateral (upper panel) and ipsilateral (lower panel) to the previously gravid uterine horn in cows injected from days 1 to 5 postpartum with a prostaglandin synthetase inhibitor (FM) and infused continuously from days 1 to 10 postpartum with $PGF_{2\alpha}$ (FM + $PGF_{2\alpha}$) or physiological saline (FM + saline) and in untreated cows (control).


CONTRALATERAL OVARY
ACCUMULATED UNITS OF CL OCCURENCE
DAYS POSTPARTUM
FM+PGF$_{2\alpha}$
FM+SALINE
CONTROL
1
2
3
4
5
6
7
8
10
20
30
40
50
60

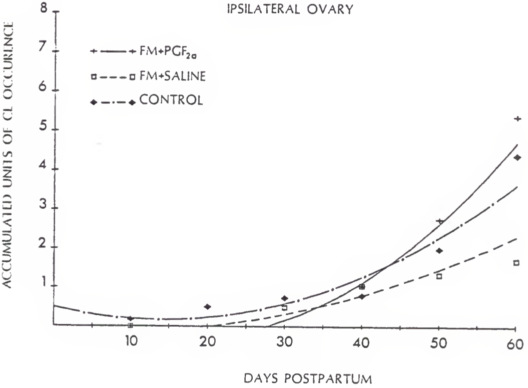

IPSILATERAL OVARY
ACCUMULATED UNITS OF CL OCCURENCE
DAYS POSTPARTUM
FM+PGF$_{2\alpha}$
FM+SALINE
CONTROL
1
2
3
4
5
6
7
8
10
20
30
40
50
60

TABLE 5.9. Within cow partial correlations of responses measured in postpartum Brown Swiss cows.

	Progesterone	Estradiol	Cervical Diameter	Uterine Horn Diameter	Location of Uterus	Ovarian Volume
PGFM	-0.04 (342)^{a}	0.11 (345)	0.48** (31)	0.36^{+} (22)	-0.57** (32)	0.19 (50)
Progesterone		0.01 (1153)	-0.44** (324)	-0.32** (662)	0.30** (322)	0.18** (713)
Estradiol			0.06 (337)	0.05 (688)	-0.02 (335)	-0.02 (739)
Cervical Diameter				0.83** (3.23)	-0.70** (330)	0.15** (340)
Uterine Horn Diameter					-0.68** (319)	-0.03 (696)
Location of Uterus						-0.20** (338)

[a] Degrees of freedom in parentheses.
** $P < 0.01$; * $P < 0.05$; + $P < 0.1$.

uterine horn diameter and in return of the uterus into the pelvic canal were highly significant ($P < 0.01$) and reflect similar pattern of changes for each of these responses. Correlations between ovarian volume and cervical diameter ($r = 0.15$; $P < 0.01$) or location of the uterus ($r = -0.20$; $P < 0.01$) were related probably to the gradual demise of the CL of pregnancy as the uterus involuted.

Discussion

In cattle, uterine involution is considered complete when the uterus has returned to its normal nonpregnant position, when the two uterine horns are similar in diameter, and both show normal consistency and tonus (Casida, 1968). Results of the present experiment indicate that two of these criteria (uterine horn diameter and location of the uterus) and cervical diameter were not affected by suppression of endogenous prostaglandins during the early postpartum period. In these three groups of cows, profiles of changes in cervical and uterine horn diameter and in location of the uterus were similar, and uterine involution was completed by approximately day 35. This is in agreement with previous estimates of 39 days for average interval from parturition to complete uterine involution (Marion et al., 1968) and within the range of 28 to 56 days in studies reviewed by Casida (1968) and Kiracofe (1980).

It appeared from results of this experiment that physiological processes involved in uterine involution such as vasoconstriction, peristaltic muscular contraction (Jordan, 1952; Venable and McDonald, 1958; Riesen, 1968; Gier and Marion, 1968) and re-organization of the connective tissue framework (Wray, 1982) could be initiated despite a significant reduction in prostaglandin production during the early postpartum period. No relationship was detected between postpartum decrease in UBF and uterine venous $PGF_{2\alpha}$, a known vasoconstrictor (see Chapter IV). After expulsion of the feto-placental unit, increased vascular resistance may be due to removal of a vasodilatory factor of conceptus origin. It has been suggested that formation of gap junctions, which are partially under the control of prostaglandins, promotes expulsive contraction at parturition by facilitating spread of action potentials between myometrial cells (Garfield et al., 1977; 1979; 1980). However, disappearance of gap junctions occurs within 24 h after parturition (Berezin et al., 1982). During the postpartum period, myometrial contractions may be brought about mainly by pituitary oxytocin (Schams et al., 1979; Hanzen, 1982; Ruesse, 1982) without any obligatory role for prostaglandins (Chan, 1977). Removal of uterine distension after fetal expulsion appears to be a preponderant stimulus of uterine involution. In the rat, even after the fetuses have been removed at term, uterine involution and collagen breakdown were prevented as long as distension of the uterus was maintained by the presence of inert material (Wray, 1982).

Although a basal endogenous production of prostaglandins does not appear to be an obligatory factor in the process of uterine involution, an augmentation of $PGF_{2\alpha}$ secretion may enhance the processes of uterine involution. Higher magnitude (Lewis et al., 1984) and longer duration (Lindell et al., 1982) of the endogenous release of $PGF_{2\alpha}$ from the uterus, as assessed by measurement of peripheral plasma PGFM concentrations, were related to faster involution of the uterus. Likewise, preliminary observations suggest that exogenous injections of $PGF_{2\alpha}$ may shorten the interval from parturition to complete uterine involution in dairy cattle (Kindahl et al., 1982). Perhaps higher $PGF_{2\alpha}$ or its continued presence postpartum provided an additional stimulus that favors smooth muscle contraction in uterine tissues as suggested by Lindell et al. (1982). Results of the present experiment indicated that an experimentally induced decrease in prostaglandin secretion did not delay uterine involution.

For 15 of the 18 cows used in this experiment, first rises in progesterone were accompanied by detection of a palpable CL and thus corresponded to the period following first ovulation. This is in agreement with previous reports (Arije et al., 1971; Echternkamp and Hansel, 1973; Stevenson and Britt, 1979) indicating that blood concentration of progesterone remained low until first ovulation in dairy cows. Detection of a palpable CL occurred only after the second P_4 rise in the three other cows (one per group). A rise in plasma progesterone concentration prior to first ovulation has been reported in beef cows (Pope et al., 1969; Donaldson et al., 1970; Lavoie and Moody, 1976; Humphrey et al., 1976; Webb et al., 1977; Lavoie et al., 1981). A possibility also exists that CL may have been undetected by rectal palpation in these three cows. Interval from parturition to first ovulation averaged 30 days and ranged from 16 to 58 days among the 15 cows that ovulated in this experiment. Studies with dairy cows as reviewed by Kiracofe (1980) indicated that first ovulations occurred approximately 14 to 22 days postpartum in a large proportion of dairy cows (Marion and Gier, 1968). In the present experiment, 67% (4/6) and 50% (3/6) of cows in the control and FM +

$PGF_{2\alpha}$ groups respectively, ovulated within the first 25 days postpartum as compared to 16.6% (1/6) in the FM + saline group.

Duration of P_4 rises may be underestimated and does not represent the exact length of the luteal phase since cows were bled thrice weekly at the time a P_4 rise occurred. However, comparison of parameters for first and second rises is relevant within the present study. Duration and magnitude of the first P_4 rise were lower than for the second rise. This is in agreement with previous reports (Menge et al., 1962; Morrow et al., 1969; Kesler et al., 1978; Stevenson and Britt, 1979; Ward et al., 1979) indicating that CL formed after first ovulation had a shorter life span and that lower plasma concentrations of progesterone were measured after first ovulation. The postpartum increase in PGFM concentrations has been proposed as one of the possible mechanisms implicated in occurrence of short luteal phases after first ovulation (Kesler et al., 1981). In such an eventuality, a longer luteal phase following first ovulation would be expected after suppression of prostaglandin synthesis early postpartum. However, in the present experiment, duration of first P_4 rise in the FM + saline group was not extended in comparison to the FM + $PGF_{2\alpha}$ or the control group (Table 5.5). In agreement with Kesler et al. (1981), present results argue against a lack of luteotrophic support as a factor involved in short luteal phases after first ovulation postpartum; lengths of first luteal phases were comparable among the three treatment groups despite higher plasma LH concentrations in the control group. Perhaps failure of luteal cells to acquire proper responsiveness to LH may be an important factor in determining the length of the first luteal phase in postpartum cows as suggested by Kesler et al. (1981). CL maintenance observed after first ovulation in two cows hysterectomized on the day of parturition (see Chapter III) indicates that the postpartum uterus has a luteolytic capacity, but does not imply that the uterus is the direct cause of short luteal phases observed after first ovulation. In intact postpartum cows, the lower progesterone concentrations resulting from inadequate corpus luteum function (Stevenson and Britt, 1979; Kesler et al., 1981) may facilitate the release of a luteolytic uterine factor, possibly $PGF_{2\alpha}$, thereby causing short luteal phases.

As indicated by rectal palpation, first ovulation occurred on the ovary contralateral to the previously gravid uterine horn in 86% of the cases in the present study. This is consistent with earlier reports (Saiduddin et al., 1967; Foote and Peterson, 1968; Marion and Gier, 1968). In the present study, only three cows exhibited estrus at first ovulation and based upon reviews by Morrow (1969) and Kiracofe (1980), this should be considered a normal event in dairy cows.

Differences detected in overall progesterone response (accumulated progesterone) over the 60 day experimental period (Figure 5.4) suggest that suppression of prostaglandin synthesis during the early postpartum period may be detrimental to restoration of ovarian function. Consistent with earlier findings (Lewis et al., 1984), criteria used to describe the process of uterine involution were correlated inversely with postpartum progesterone concentrations. However, the involuting uterus does not appear to be the cause of differences detected in accumulated progesterone concentrations since the rates of uterine involution were not different between groups. Alternatively, early postpartum suppression of prostaglandin synthesis may have altered either central (hypothalamic and/or pituitary), utero-ovarian, and/or intraovarian control of ovarian activity.

It is possible that a lower overall postpartum P_4 response may be due to decreased hypothalamic function early postpartum in both groups that received FM. A reduced frequency of pulsatile gonadotropin-releasing hormone (GnRH) secretion from the hypothalamus (Walters et al., 1982) appears to be the cause of low pulsatile and low basal concentrations of circulating LH in postpartum beef and dairy cows that are suckled (Humphrey et al., 1976; Stevenson and Britt, 1979; Forrest et al., 1979; Carruthers and Hafs, 1980; Webb et al., 1980; Rawling et al., 1980; Walters et al., 1982). Prostaglandins (E and F series) may cause gonadotropin secretion by stimulating GnRH release from the hypothalamus (Spies and Norman, 1973; Harms et al., 1974; Chobsieng et al., 1975; Drouin et al., 1976; Labrie et al., 1976; Roberts et al., 1976). Nonspecific suppression of prostaglandin secretion with FM may have altered hypothalamic function which would account for the reduced LH concentrations in both the FM + saline and FM + $PGF_{2\alpha}$ groups compared to the control group (Figure 5.5). A physiological replacement with a constant infusion of only $PGF_{2\alpha}$ did not restore plasma LH concentrations in the FM + $PGF_{2\alpha}$ group, although earlier reports indicated that acute injection causes the release of LH (Haynes et al., 1978; Bono et al., 1980).

Richards and Midgley (1976) proposed that just prior to antrum formation, follicles develop the ability to produce estrogens which makes the follicle more responsive to gonadotropins which in turn further increases estrogen production. This synergistic effect of gonadotropin and estrogens would allow the follicle to enter a rapid growth phase. A positive relationship between estrogen and LH secretion, which appeared to be of physiological importance in follicular development prior to first ovulation, has been reported in dairy cows (Stevenson and Britt, 1980). By decreasing LH (Figure 5.5) and perhaps FSH secretion, early postpartum suppression of prostaglandin synthesis may have altered the positive interaction of gonadotropins and estrogens and lead to the lower estrogenic activity in both groups treated with FM (Figure 5.6).

The possibility exists that suppression of intraovarian prostaglandin synthesis may have altered ovarian follicular development early postpartum. There is an ovarian competence for follicular activity during the early postpartum period (Foote and Peterson, 1968; Marion and Gier, 1968; Saiduddin et al., 1968; Wagner and Hansel, 1969; Stevenson et al., 1983). Recent histological findings demonstrated that there was a complete depletion of small antral follicles (0.16 to 0.28 mm diameter) that accompanied a gradual replenishment of larger follicles (0.29 to 0.67 and 0.68 to 1.57 mm classes) between days 15 and 35 postpartum (Dufour and Roy, 1983). Moreover, estimates of follicular growth, from antrum formation to preovulatory size, were about 45 and 41 days in sheep (Turnbull et al., 1977) and cows (Lussier et al., 1983), respectively. Thus, the first ovulation postpartum involved follicles that were recruited from a pool of growing follicles early postpartum. Follicular tissues (both granulosa and theca cells) were capable of producing substantial amounts of prostaglandins (Plunkett et al., 1975; Triebwasser et al., 1978; Evans et al., 1983; Poyser, 1983) which raised the possibility that intraovarian prostaglandins may play a role in follicular development (Armstrong, 1981). It is believed that cAMP mediates the actions of gonadotropins (Funkenstein et al., 1980). However, prior to acquisition of responsiveness to gonadotropins, prostaglandins, mainly of the E series can induce an increase in cAMP (Armstrong, 1981). Therefore, the possibility exists that prostaglandins modulate early stages of granulosa cell differentiation by regulating cAMP-dependent protein kinase and the phosphorylation of specific intracellular proteins in developing follicles (Richard et al., 1983). Thus during the early postpartum period, at a time when the ovary is "re-programmed into its cyclic mode", suppression of prostaglandin synthesis may have altered follicular growth and differentiation. Decreased overall luteal function observed in the FM + saline group may have been due to growth of less competent follicles with lower responsiveness to gonadotropin or a general delay in follicular growth. Tam and Roy (1982) provided evidence that $PGF_{2\alpha}$ was required and had a direct action on follicular growth in the guinea pig. Therefore, small antral follicles exposed to exogenous $PGF_{2\alpha}$ (FM + $PGF_{2\alpha}$ group) during the early postpartum period may have regained their competency which is later expressed by a gradual increase in luteal function (Figure 5.4).

Following luteal regression during the estrous cycle, both estrogen-active (nonatretic) and estrogen-inactive (atretic) follicles are present on ovaries (Ireland and Roche, 1983). The large variation in plasma estrogen concentrations of samples collected at frequent intervals (Eley, 1980) may reflect growth and atresia of follicles as suggested previously (Echternkamp and Hansel, 1973; Fernandes et al., 1978a; Stevenson and Britt, 1979). Likewise, the anovulatory postpartum period in cattle is characterized by increased follicular growth and a coincident increase in the ability of the largest follicle to produce estradiol (Spicer et al., 1983). Decrease in variability and in rate of accumulation of estradiol early postpartum (less than 25 days postpartum; FM + $PGF_{2\alpha}$ and FM + saline) may indicate a potential role for prostaglandins for steroidogenesis in large antral follicles. Since both the FM + saline and FM + $PGF_{2\alpha}$ groups were equally affected, prostaglandins other than $PGF_{2\alpha}$ may be involved in follicular steroidogenesis. This is at variance with previous observations indicating that no functional correlates could be established in vitro between production of steroid and prostaglandins in prepuberal pig ovaries (Evans et al., 1983).

Enhanced luteal function in the FM + $PGF_{2\alpha}$ group was associated with greater ovarian activity of ovaries ipsilateral to the previously gravid uterine horn. Lewis et al. (1984) reported that when cows were heat stressed during the last trimester of gestation, they had higher plasma PGFM concentrations postpartum and a greater ovarian activity on ovaries ipsilateral to the previously gravid uterine horn. Collectively, these results suggest that the uterus and mainly the previously gravid uterine horn may affect ovarian follicular development via release of $PGF_{2\alpha}$ into the ovarian circulation. The CL of pregnancy was palpable, and its size reduced steadily for about 2 weeks postpartum (Wagner and Hansel, 1969). Although the CL of pregnancy does not prevent follicular development (Marion and Gier, 1968; Wagner and Hansel, 1969; Moller, 1970), the regressing CL of pregnancy may have an immediate or carryover effect on the ovary (Foote and Peterson, 1968). Time interval for follicles to develop to half mature size was appreciably shorter on ovaries that did not contain the CL of pregnancy (Casida and Venzke, 1936). Higher peripheral PGFM concentrations postpartum were associated with a more rapid decrease in ovarian volume of ovaries ipsilateral to the previously gravid uterine horn (present study) and a smaller diameter for the regressing CL of pregnancy (Lewis et al., 1984). Thus the higher release of $PGF_{2\alpha}$ by the postpartum uterus may cause a faster demise of the CL of pregnancy which would promote follicular growth and contribute to higher ovarian activity in ovaries ipsilateral to the previously gravid uterine horn. Recent observations indicated that a local exchange of $PGF_{2\alpha}$ from uterine venous plasma to ovarian arterial plama was possible in postpartum cattle (D.B. Foster, unpublished observations).

A specific enhanced response of ovaries, ipsilateral to the previously gravid uterine horn, to $PGF_{2\alpha}$ may be attributed also to alteration of ovarian blood flow which is critical to proper ovarian function (Dufour et al., 1971; Niswender et al., 1976; Ford and Chenault, 1981; Wise et al., 1982). In cattle, an anastamosis between uterine and ovarian blood supplies via the uterine branch of the ovarian artery (UBOA; Mapletoft et al., 1976) allows flow of blood in both directions depending on stage of the estrous cycle (Lamond and Drost, 1974; Ford and Chenault, 1981). Thus, during the luteal phase of the estrous cycle, at which time vascular resistance is increased in the uterus, the uterine artery may contribute arterial blood to the CL-bearing ovary via the UBOA (Ford and Chenault, 1981). During pregnancy, blood flow to the uterus increases steadily until 200 days of gestation (Ferrel and Ford, 1980), and the UBOA enlarges significantly during the same period of time (Lamond and Drost, 1974). Presumably, flow of blood in the uterine branch of the ovarian artery would be directed toward the uterus during pregnancy since vascular resistance is minimal during that period (Greiss and Anderson, 1974; Rosenfeld et al., 1974; 1976). In nonpregnant and early pregnant monkeys, uterine arterial blood supplies most of the blood to the ovaries, whereas during late pregnancy the ovarian artery is the major source of blood to the ovaries (Wehrenberg et al., 1977). Thus, following expulsion of the feto-placental unit, increased uterine vascular resistance and a decrease in uterine blood flow (see Chapter IV) may be associated with a significant shunt of blood of uterine origin to the ovary via the UBOA. As proposed by Lamond and Drost (1974), an enhanced postpartum blood supply to the ovary may exceed its needs and become incompatible with proper ovarian function. Perhaps $PGF_{2\alpha}$ of uterine origin also regulates postpartum ovarian function by causing vasoconstriction of the UBOA thereby contributing to reestablishment of an adequate blood flow to the ovary ipsilateral to the previously gravid uterine horn. Lack of such type of control in the FM + saline group may have been detrimental to ovarian activity of ovaries ipsilateral to the previously gravid uterine horn. Lamond and Drost (1974) stated that the UBOA has a wide capacity for adjusting flow rates. Present results suggest that $PGF_{2\alpha}$ may be involved in control of blood flow to ovaries ipsilateral to the previously gravid uterine horn since replacement with $PGF_{2\alpha}$ specifically increased ovarian activity of this ovary in the FM + $PGF_{2\alpha}$ group.

Although $PGF_{2\alpha}$ did not appear to have an obligatory role in the process of uterine involution, its suppression early postpartum had deleterious effects on ovarian function. Despite the fact that this experiment was not designed to investigate specific mechanisms, results suggest that early postpartum release of uterine $PGF_{2\alpha}$ may be important in reestablishment of ovarian function.

CHAPTER VI
PERIPARTURIENT ENDOCRINE CHANGES OF CONCEPTUS AND MATERNAL UNITS IN HOLSTEIN HEIFERS BEARING GENETICALLY DIFFERENT CONCEPTUSES

Introduction

Classical experiments involving reciprocal crosses between either different breeds of horses (Walton and Hammond, 1938) or cattle (Joubert and Hammond, 1958) that were markedly different in size, illustrated the importance of the maternal environment in regulating fetal growth. Conversely, the conceptus (fetus, placenta and fetal fluids) influences the maternal unit during pregnancy when such phenomena as maternal recognition of pregnancy (Northey and French, 1980), placentation (Amoroso, 1952), mammary gland development (Tucker, 1969) and parturition (Holm, 1967; Kennedy, 1971) are considered.

Both conceptus and maternal units undergo morphological and endocrine changes in order to adapt to certain environmental conditions. For example, after physically altering placental development, in sheep (Alexander, 1964; Emmanouilides et al., 1968; Hobel et al., 1970; Metcalfe, 1970; Cefalo et al., 1977; Clapp et al., 1981; Caton et al., 1983), compensatory growth of fetal and/or maternal parts of the placentome reflected a degree of interaction between maternal and conceptus units that was induced in an attempt to provide optimal conditions for fetal development. In cows that were heat stressed during the last trimester of gestation, certain prepartum maternal concentrations of hormones of maternal (progesterone) or of conceptus (estrone sulfate)origin (Collier et al., 1982) and postpartum PGFM concentrations (Lewis et al., 1984), which reflected uterine production of F series prostaglandins (see Chapter III and IV), were altered. Therefore, the environment in which the fetus grows may not only be subjected to maternal regulation but also to conceptus regulation since both units interact during gestation.

The service sire has been implicated as a factor controlling variability in gestation length (Defries et al., 1959; Boyd and Hafs, 1965), calf birth weight (Joubert and Hammond, 1958; Boyd and Hafs, 1965), and milk production (Adkinson et al., 1977) in cattle. Furthermore, Osinga (1978) reported that sire of fetus or breed of sire of fetus influenced the urinary creatinine/estrogen ratio during late pregnancy. This suggests that the endocrine balance of the maternal unit during pregnancy may be affected also by genotype of the conceptus. In a previous experiment with Jersey cows (Eley et al., 1981a; 1981b), selection for milk yield influenced various physiological, hormonal and production responses of the maternal unit. However, the ability to clearly test the effect of sire of fetus on responses of the maternal unit was compromised by the fact that sire of fetus and sire of dam effects were confounded.

The present experiment was designed to delineate sire of fetus effects upon prepartum and postpartum endocrine responses of the maternal unit and to further test the hypothesis that genotype of fetus influences hormonal balance of the dam (Eley et al., 1981a). This was accomplished by determining prepartum and postpartum hormonal changes of the maternal unit that were associated with pregnancies in

Holstein heifers initiated by three genetically different service sire-breed groups (e.g., Holstein, Brahman, and Angus service sire).

Materials and Methods

Experimental Design and Management

Twenty-four virgin Holstein heifers (age: 13-17 months; $\overline{X}$ = 14.4 ± 0.6 months), sired by four purebred Holstein bulls, were assigned to three service sire-breed groups. Heifers (n = eight per service sire-breed group) were inseminated artifically to either purebred Holstein, Brahman or Angus bulls. Distribution of Holstein heifers to each service sire group is presented in table 6.1. Assignment of Holstein heifers to each service sire-breed group was arranged so that sire of heifer effects (four sires) were balanced across service sire-breed groups. This was done by giving equal representation to sire of heifer in each service sire-breed group. Thus, sire-breed of fetus and sire of heifer effects were not confounded. Within each service sire-breed group, four different bulls were utilized for insemination. Therefore, these three groups of uniform Holstein hiefers bore genetically different conceptuses (i.e., purebred Holstein x Holstein or crossbred Holstein x Brahman or crossbred Holstein x Angus). With this type of experimental design, measurement of maternal responses provided a sensitive estimate of conceptus genotype effects due primarily to breed of the service sire.

Two heifers, assigned to the Holstein and Angus service sire groups, failed to conceive within 7 months from the beginning of the experiment; one heifer assigned to the Brahman service sire group aborted in mid pregnancy. These three heifers were removed from the experiment. Therefore, 21 Holstein heifers (n = seven per service

TABLE 6.1. Experimental design for assignment of Holstein heifers (H-heifer number) to Holstein, Brahman, and Angus service sire groups. Sire of heifer (A, B, C and D) was equally represented in each group. Number in parentheses gives identification of bull used for artifical insemination.

	Service sire-breed group		
Sire of heifer	Holstein	Brahman	Angus
A	H-765[a] (1) H-782 (2) H-800 (3)	H-778 (5) H-780 (6) H-798 (7)	H-770 (9) H-779 (10) H-801 (11)
B	H-767 (4) H-781 (1) H-789 (2)	H-772 (8) H-783 (5) H-784 (6)	H-773 (12) H-777 (9) H-787[a] (10)
C	H-794 (3)	H-792[b] (7)	H-775 (11)
D	H-788 (4)	H-793 (8)	H-791 (12)

[a] Failed to conceive after four inseminations and were removed from experiment.

[b] Aborted in mid-pregnancy and heifer was removed from experiment.

sire-breed group) were included in this study. In the following text, tables and figures, groups of Holstein heifers bearing Holstein x Holstein, Holstein x Brahman and Holstein x Angus conceptuses will be referred to as Holstein, Brahman and Angus service sire-breed groups, respectively.

Breeding of heifers was initiated on October 16, 1979, and heifers in the three service sire-breed groups had contemporary calvings from June 21 to December 22, 1980. To limit effects of heat stress during the summer months, heifers were given free access to a large shade structure (Roman-Ponce et al., 1977) from May 1, 1980 until the end of the experimental period. Heifers were fed a basal ration consisting of corn silage, Bermuda grass hay and cotton seed hulls mixed with concentrate. Approximative quantities of basal ration and concentrate given to each heifer were 8 and 1 kg, respectively, during gestation and 18 and 6 kg during lactation.

Blood Collection

Collection of blood samples started on day 160 of pregnancy for each animal. Blood samples were collected thrice weekly (Monday, Wednesday, and Friday) from day -120 to -16 prepartum (day 0 = expected day of parturition) and from day 16 to 60 postpartum. During the periparturient period, blood samples were collected daily from day -15 prepartum to day 15 postpartum. Blood (20 ml) usually was collected between 0800 h and 1000 h by jugular venipuncture into cold heparinized (100 i.u./tube) vacutainer tubes that were placed immediately in an ice-bath after collection. Within 1 h after blood samples were collected, hematocrit was determined in approximately 40 ul of blood using a microhematocrit centrifuge (International microcapillary, centrifuge Model MB, Needham, MA) and microcapillary tube reader (International microcapillary, reader, Needham, MA). A portion of the plasma derived from each hematocrit determination was used to estimate concentrations (g/100 ml) of plasma protein by refractometry (PP; Atago refractometer "N", Atago Co., Tokyo, Japan). The remainder of each blood sample was centrifuged at 4 C (5000 x g) for 15 min, plasma harvested and stored at -20 C until subsequent analyses. Calf birth weight, viability and sex were recorded at parturition and gestation length calculated. Milk weights were recorded twice daily following parturition.

Assay Procedures

Plasma samples were analyzed by radioimmunoassay for progesterone, estrone, estradiol, estrone sulfate, luteinizing hormone (LH) and 15-keto-13,14-dihydro $PGF_{2\alpha}$ (PGFM). To minimize variation between assays, an equal number of contemporary blood samples from each service sire-breed group was included in each assay. All estimates were done in duplicate in volumes that allowed mass of a given hormone to be within the range of the standard curve.

Progesterone concentration was measured in plasma samples collected between day -23 prepartum and day 60 postpartum. In this chapter, only prepartum progesterone concentrations from day -23 to day 0 are presented. Postpartum plasma progesterone and LH concentrations from days 1 to 60 will be presented in Chapter VII. A description and validation of the assay procedure for progesterone have been reported previously (Knight et al., 1977; Eley et al., 1981a). The antiserum (gift from Dr. J.L. Fleeger, Texas A and M University) was prepared in rabbits against 11_{α}-hydroxy-progesterone hemisuccinate conjugated to bovine serum albumin. Prepartum samples (50 and 100 ul) were extracted with a mixture (2 ml) of benzene:hexane (1:2) as described by Louis et al. (1973). Separation of bound and free labeled progesterone was done by centrifugation after addition of dextran-coated charcoal. Recovery of labeled progesterone after extraction was 92%. All prepartum samples were analyzed in one large assay that included thirteen standard curves. Repeated measurements of a reference sample ($\bar{X}$ = 4744 ± 379 pg/ml) included in duplicate in each curve resulted in an intra-assay coefficient of variation of 7.18%.

Plasma estrogens (estrone, estradiol and estrone sulfate) concentrations were determined on samples collected between day -23 prepartum and parturition (day 0) by methods previously described and validated in our laboratory (Chenault et al., 1975; Eley et al., 1981a). Estrone and estradiol were measured after extraction with diethyl ether and isolation by Sephadex LH-20 column chromatography. Antiserum used in assay was prepared in sheep against estra-1,3,5(10)-triene-3,17 - diol,17-succinyl-bovine serum albumin (gift from Dr. V.L. Estergreen, Washington State University, WA). Individual recovery of labeled estrone and estradiol were calculated and averaged 90.3 ± 1.1% and 86.6 ± 0.9%. Repeated measurements of a reference plasma samples collected from an ovariectomized cow resulted in intraassay coefficient of variation of 25.1% for estrone and 13.1% for estradiol, whereas interassay coefficient of variation were 13.4% and 18.6% respectively. After extraction of free estrogen (estrone and estradiol), aqueous phase was recuperated and estrone sulfate concentrations were determined following hydrolysis with a sulfatase enzyme (Sulfatase Type H-II, Sigma Chemical Co., St. Louis, MO). Liberated estrone was extracted with diethyl ether isolated via LH-20 column chromatography and quantified by RIA as described and validated by Eley et al. (1981a). Individual recovery of estrone sulfate was calculated and averaged 72.4 ± 1.3%. Intra- and interassay coefficient of variation of a reference plasma samples from an ovariectomized cow were 27.7% and 30.36%, respectively.

Concentrations of 15-keto-13,14-dihydro $PGF_{2\alpha}$ (PGFM) were measured in unextracted plasma samples obtained from day -2 prepartum to day 15 postpartum. Procedure and validation of the assay has been described in Chapter III. The antiserum (gift from Dr. K. Kirton, The Upjohn Company, Kalamazoo, MI) was prepared in goat against 15-keto-13,14-dihydro $PGF_{2\alpha}$ conjugated at the C_1 carbon to serum albumin by the carbodiimide method (Cornette et al., 1974). Unextracted plasma samples were assayed, and separation of free and bound PGFM was done by protein precipitation with polyethylene glycol-6000. Thirty-three measurements of a reference plasma sample ($\overline{X}$ = 531.6 ± 65.8 pg/ml) in eight assays resulted in intra- and interassay coefficients of variation of 11.8% and 1.8% respectively.

Statistical Analyses

Least squares analyses of variance of periparturient endocrine responses were conducted utilizing the General Linear Models procedure of the Statistical Analysis System (SAS; Barr et al., 1979). Experimental design was in the nature of a split split plot in which service sires were nested in breed (of service sire) and heifers were nested in service sire and in breed. Repeated measurements over time were taken on heifers, and day was considered as a continuous independent variable. Mathematical model and proper error term for each source of variation considered is given in table 6.4. Each response was characterized by a day trend which was analyzed by polynomial regression. Tests of homogeneity of regression were used to detect differences in day trends for periparturient endocrine responses between service sire-breed groups. In addition, when service sire-breed group response curves were not parallel ($P < 0.05$), orthogonal contrasts of service sire groups response curves were made as follows: Holstein, Brahman versus Angus; Holstein versus Brahman.

Results

General

All heifers used in this experiment had a normal pregnancy, and labor was initiated at approximately the expected time of delivery. Close to parturition, heifers were examined for calf size and viability (i.e., response of the fetus to manual stimulation via rectal palpation of the dam). Gestation length, conditions of parturition, calf birth weight and sex, and postpartum uterine status for each heifer are presented in Appendix C (Table C.1). In all cases, calves were viable and process of parturition appeared to have been initiated normally. In the case of one heifer in the Brahman service sire group, a decision was made to perform a cesarian section at 285 days of gestation after labor had been initiated. Calf birth weight in this case was 68 kg.

Dystocia was more frequent in the Brahman service sire group, and including the cesarian section, four of the seven heifers required assistance during parturition. One heifer in each of the Holstein and Angus service sire groups had dystocia.

Although all calves were viable during the process of labor, one, two and three calves were stillborn in the Brahman, Angus and Holstein service sire groups, respectively. In all cases of stillbirth, parturition occurred on approximately the expected day of calving, and physical appearance of the stillborn calf was normal. Postmortem examination of stillborn calves did not reveal evidence for bacterial infection or pulmonary deficiency and cause of stillbirth remains unknown. Hormonal data (estrone, estradiol, estrone sulfate and PGFM) collected from heifers that had clinical problems (dystocia, stillbirth and uterine infection) were analyzed separately as a group (n = 10) and compared to those heifers experiencing a normal parturition or postpartum period (n = 11). No significant differences in day trends were detected ($P > 0.1$) except for estrone sulfate (Appendix C; Table C.2). This difference was interpreted as being due mainly to a different profile of estrone sulfate in heifers assigned to the Brahman service sire group. When analysis of estrone sulfate was performed only on heifers of the Angus and Holstein service sire groups that were regrouped as clinically normal (n = 8) or abnormal (n = 6), there was no difference ($P > 0.1$) in day trend of estrone sulfate. This suggests that subsequent differences to be described between service sire-breed groups were real and were not due only to clinical disturbances. Therefore, results presented in this report included data from all heifers assigned to the three service sire-breed groups.

Mean gestation length was longer ($P < 0.05$) in the Brahman service sire group than in Holstein or Angus service sire groups (285.0 ± 3.2 versus 279.7 ± 1.3 and 279.9 ± 2.1 days, respectively; Table 6.2). Mean calf birth weight of Holstein heifers bred to Angus bulls was lower ($P < 0.05$) than that of Holstein heifers bred to either Holstein or Brahman bulls (30.6 ± 1.4 versus 36.0 ± 1.6 and 43.4 ± 4.6 kg, respectively; Table 6.2).

Statistical analysis and illustrations of prepartum and postpartum profiles of changes in hematocrit and plasma protein are presented in Appendix C (Tables C.3 and C.4; Figures C.1 and C.2) and are consistent with earlier reports (Eley et al., 1981a; Collier et al., 1982; Lewis et al., 1984). In the three service sire-breed groups, hematocrit increased prior to and decreased after parturition and protein concentrations were higher postpartum than prepartum.

Endocrine Responses

Prepartum and postpartum least squares means and analysis of variance of dependent variables for Holstein, Brahman and Angus service sire groups are presented in Tables 6.3 and 6.4, respectively. During the prepartum period, no differences between overall means for service sire-breed groups were detected ($P > 0.1$) in any of the responses measured. During the postpartum period, overall mean concentrations of PGFM were lower in the Angus service sire group than in the Holstein and Brahman service sire groups.

Polynomial regression equations describing daily changes in hormonal responses are presented in Appendix C (Tables C.5 and C.6). Test of homogeneity of regression for each response studied is given in Table 6.5. Probability value of less than 0.05 indicates that the pattern of daily changes in a given response differs between service sire-breed groups. Day trends of progesterone concentrations were not parallel between groups ($P < 0.05$; Table 6.5; Figure 6.1). Between day -21 and -3 prepartum, progesterone concentrations decreased gradually in the three service sire-breed groups. However, during

TABLE 6.2. Means (± S.E.M.) of gestation length and calf birth weight for Holstein heifers bred to either Holstein, Brahman or Angus bulls.

	Service sire-breed group		
Responses	Holstein	Brahman	Angus
Gestation length (days)	278.7 ± 1.3[a]	285.0 ± 3.2[b]	279.0 ± 2.1[a]
Calf birth weight (kg)	36.1 ± 1.6[a]	43.4 ± 4.6[a]	30.6 ± 1.4[b]

[a,b] Group mean value with different superscripts differ ($P < 0.05$).

TABLE 6.3. Least squares means of periparturient endocrine responses in Holstein heifers bred to either Holstein, Brahman or Angus bulls.

	Service sire-breed group			S.E.M.[c]
	Holstein	Brahman	Angus	
Prepartum response				
Progesterone (ng/ml)	4404	4734	4087	± 374
Estradiol (pg/ml)	124	129	112	± 20
Estrone (pg/ml)	843	897	693	± 136
Estrone sulfate (pg/ml)	12642	13902	8772	± 1407
Postpartum response				
PGFM[d] (pg/ml)	912[a]	1052[a]	551[b]	± 120

[a,b] Group means with different superscripts are significantly different ($P < 0.05$).

[c] Standard error of means; they are approximate, conservative, and similar for each service sire-breed group.

[d] PGFM = 15-keto-13,14-dihydro $PGF_{2\alpha}$; measured from day -2 prepartum to day 15 postpartum.

TABLE 6.4. Least squares analyses of variance of periparturient endocrine responses in Holstein heifers bred to either Holstein, Brahman or Angus bulls.

	Progesterone		Estrone		Estradiol		Estrone sulfate		PGFM		
	df	MS	df	MS	df	MS	df	MS	df	MS	Error term
Breed	2	170.50	2	14.82	2	0.22	2	10401	2	92.69**	Service sire (breed)
Service sire (breed)	9	234.59	9	26.12	9	0.78*	9	3850*	9	6.25	Heifer (service sire,breed)
Heifer (service sire, breed)	9	124.32**	9	16.30**	9	0.23*	9	1065**	9	31.93	Residual
Days[a]	3	1951.00**	3	317.00**	3	4.86**	3	5986**	3	655.00**	Residual
Residual	351	11.55	328	1.92	300	0.08	349	182	385	19.84	

df = degrees of freedom; MS = MS x 10^5 = mean square.

[a] See Table 6.5 for tests of homogeneity of regression.
** P < 0.01; * P < 0.05.

that period, progesterone concentrations were maintained approximately 1.0 ng/ml higher in the Holstein and Brahman service sire groups than in the Angus service sire group until the time of luteolysis when progesterone concentrations decreased to similar values in all groups ($P < 0.01$; Table 6.5).

Profiles of prepartum changes in free estrogens (estrone and estradiol) were not parallel between groups ($P < 0.01$; Table 6.5). Until the last 10 days prepartum, plasma concentrations of estrone increased similarly in the three groups but profiles of estrone concentrations diverged between groups thereafter (Figure 6.2). During the last 10 days before parturition, estrone concentrations increased from mean values of 750 on day -10 to 2000 and 1500 pg/ml on day -1 in the Brahman and Holstein service sire groups, respectively; the increases in estrone concentrations were not parallel ($P < 0.01$; Table 6.5) between these two groups. Concurrently, concentrations of estrone were consistently lower in the Angus service sire group which also had a slower prepartum rise that never exceeded 1100 pg/ml ($P < 0.01$; Table 6.5; Figure 6.2). Concentrations of estradiol were approximately 10% those of estrone (Table 6.3). Profiles of estradiol concentrations essentially paralleled those of estrone. Until day -8 prepartum, estradiol concentrations were similar between groups but diverged thereafter ($P < 0.01$; Table 6.5; Figure 6.3). Prior to parturition, there was a sharper increase in estradiol concentrations in the Brahman than in the Holstein service sire group and the profile of estradiol concentrations was lower in the Angus service breed group.

TABLE 6.5. Tests of homogeneity of regression and orthogonal contrasts of periparturient endocrine responses in Holstein heifers bred to either Holstein, Brahman or Angus bulls.

	Order of regression	Error term		Treatment		Orthogonal contrast[c]	
		df[a]	MS[a]	df[b]	MS_D[b]	A	B
Prepartum response							
Progesterone	3	345	1124318	6	2902055*	**	NS
Estrone	3	322	166098	6	1579837**	**	**
Estradiol	3	294	7481	6	39684**	**	**
Estrone sulfate	3	343	15700064	6	165841084**	**	**
Postpartum response							
PGFM	3	379	1223637	6	5837104**	**	NS

[a] Degrees of freedom (df) and mean squares (MS) associated with the error term when individual curves were generated for each treatment.

[b] Degrees of freedom (df) and mean square (MS_D) associated with the difference in residuals sums of squares between fitting one pooled curve versus an individual curve for each treatment.

[c] Orthogonal contrast: A = Holstein, Brahman versus Angus
B = Holstein versus Brahman.

** P < 0.01; * P < 0.05; NS, not significant.

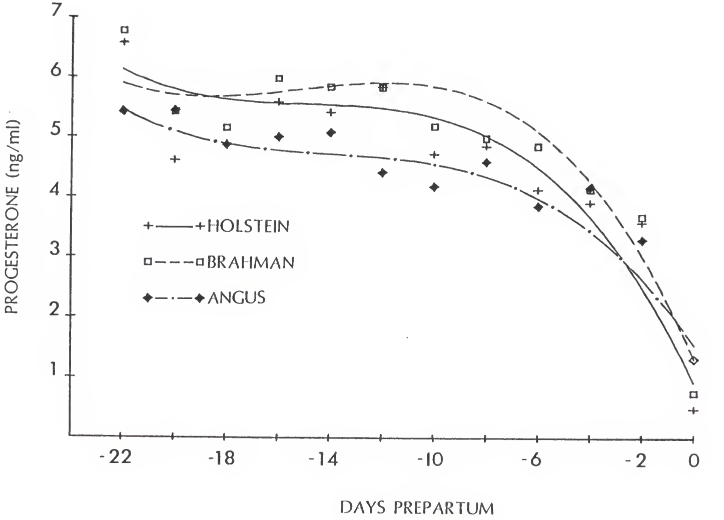


Figure 6.1 Least squares regressions and least squares means (every second day) of prepartum (day -23 to day 0) concentrations of progesterone in Holstein heifers bred to either Holstein, Brahman or Angus bulls.

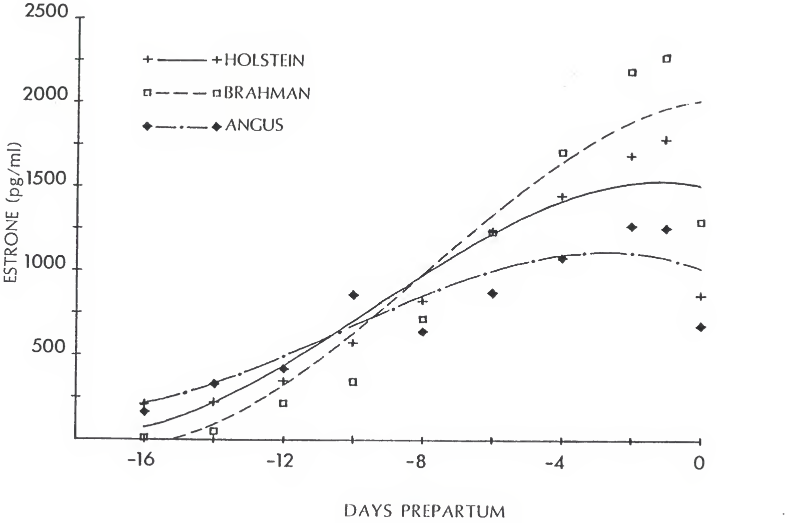


Figure 6.2 Least squares regressions and least squares means (every second day) of prepartum (day -23 to day 0) concentrations of estrone in Holstein heifers bred to either Holstein, Brahman or Angus bulls.

Differences in prepartum concentrations of estrone and estradiol between service sire-breed groups were reflected also in profiles of concentrations of estrone sulfate. Mean prepartum concentrations of estrone sulfate were 12642, 13902 and 8772 pg/ml (S.E.M = ± 1407) for Holstein, Brahman and Angus service sire groups, respectively, but these differences were not significant ($P > 0.1$; Tables 6.3 and 6.4). Prepartum day trends of estrone sulfate differed significantly among the three service sire-breed groups ($P < 0.01$; Table 6.6; Figure 6.4). Holstein heifers bearing crossbred Angus conceptuses had consistently lower estrone sulfate response curves, whereas those bearing either purebred Holstein or crossbred Brahman conceptuses had a greater rise and magnitude of estrone sulfate concentration in maternal plasma. As for the free estrogens (estrone and estradiol), the increase in estrone sulfate concentrations was even more dramatic in the Brahman than in the Holstein service sire group ($P < 0.01$; Table 6.5; Figure 6.4).

A unique trait of estrone sulfate concentration, the major product of conceptus origin present in maternal plasma (Hoffmann et al., 1976; Robertson and King, 1979; Eley et al., 1979; Eley et al., 1981a) and of estradiol was the significant effect of the service sire within breed source of variation ($P < 0.05$; Table 6.4). This suggests that within breed, mean concentrations of estrone sulfate and estradiol were different between service sires and reflect variability due specifically to the sire of fetus. Also of interest was the fact that within each service sire-breed group, appreciable variability was detected in estrone sulfate concentrations among heifers (Table 6.6). For each service sire-breed group, intraclass correlations, which

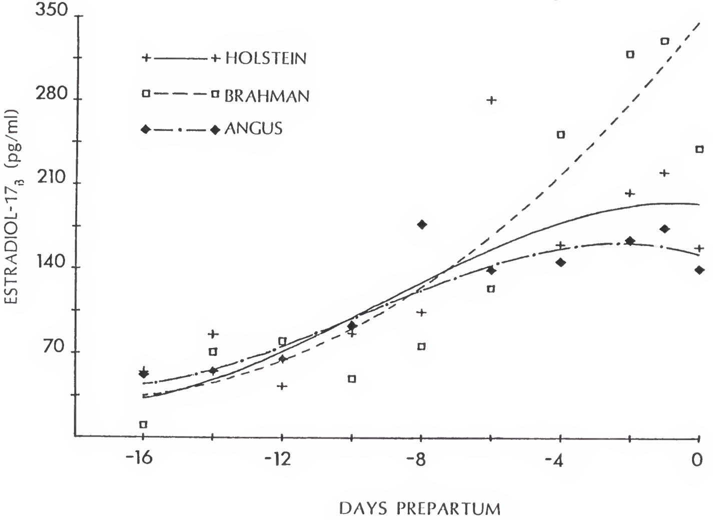


Figure 6.3 Least squares regressions and least squares means (every second day) of prepartum (day -16 to day 0) concentrations of estradiol in Holstein heifers bred to either Holstein, Brahman or Angus bulls.

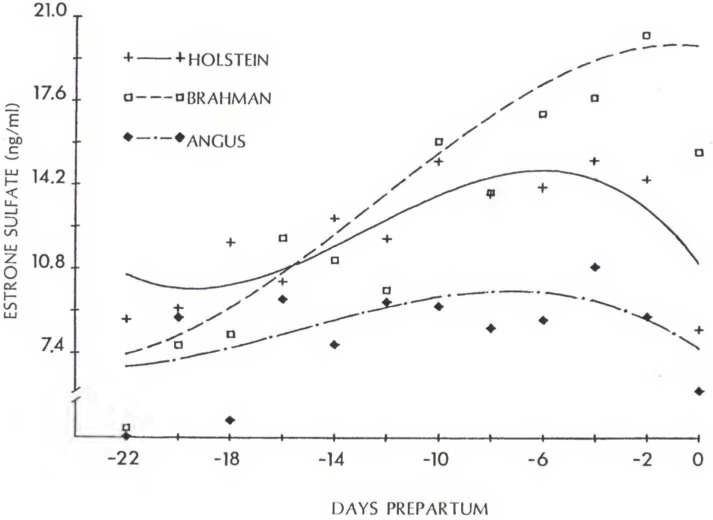


Figure 6.4 Least squares regressions and least squares means (every second day) of prepartum (day -23 to day 0) concentrations of estrone sulfate in Holstein heifers bred to either Holstein, Brahman or Angus bulls.

TABLE 6.6. Intraclass correlation coefficients of prepartum estrone sulfate concentrations in Holstein heifers bred to either Holstein, Brahman or Angus bulls.

	Service sire-breed group		
Intraclass correlation (%)	Holstein	Brahman	Angus
Unadjusted for service sire	27.4	50.3	41.8
Adjusted for service sire	25.2	21.0	37.2

[a] Intraclass correlation coefficient = σ^2 heifer/(σ^2 heifer + σ^2 residual); σ^2 = variance.

represent percent of variation (random and heifer variation) that was due to heifer, were calculated from mathematical models non-adjusted and adjusted for service sire variability (Table 6.6). Prior to adjustment for service sire variability, intraclass correlations were higher for Holstein heifers bearing crossbred conceptuses (e.g., Brahman and Angus service sire groups) than in those bearing purebred conceptuses (e.g., Holstein service sire group). However, after adjustment for service sire variability, intraclass correlations in the Brahman and Angus service sire groups were similar to intraclass correlation of heifers in the Holstein service sire group. Thus, most of the increase in variability among Holstein heifers bearing crossbred conceptuses was due to the service sire. This suggests that variation in estrone sulfate concentrations among Holstein heifers is increased when conceptus and maternal units are genetically dissimilar.

On a within heifer basis, prepartum free (estrone and estradiol; $P < 0.01$) and conjugated (estrone sulfate; $P < 0.05$) estrogens were closely interrelated and were correlated negatively with progesterone (Table 6.7). Among the 21 heifers, positive gross correlations were detected ($P < 0.01$; Table 6.7) between calf birth weight and estrone ($r = 0.57$), estradiol ($r = 0.59$) and estrone sulfate ($r = 0.64$).

Mean postpartum concentrations of PGFM differed among the three service sire-breed groups ($P < 0.01$; Tables 6.3 and 6.4). Mean PGFM concentration was 551 pg/ml for the Angus service sire group and was lower ($P < 0.01$; Table 6.4) than in Holstein and Brahman service sire groups (912 and 1052 pg/ml, respectively; S.E.M. = ± 120). Likewise, response curves of postpartum PGFM concentrations differed ($P < 0.01$; Table 6.5; Figure 6.5). Holstein heifers bearing crossbred Angus conceptuses had a lower postpartum PGFM response curve than Holstein heifers bearing either purebred Holstein or crossbred Brahman conceptuses. Postpartum profiles of PGFM concentrations for Holstein heifers bred to Holstein or Brahman bulls did not differ ($P > 0.1$; Table 6.5; Figure 6.5). Among the 21 heifers, mean PGFM concentrations were correlated positively ($P < 0.01$) with calf birth weight ($r = 0.56$; Table 6.7).

Discussion

Various responses of Holstein heifers were altered by genotype of the conceptus they bore. Mean gestation length for breeds of Holstein, Brahman and Angus cows bearing purebred fetuses were 280.0, 292.7 and 279.4 days, respectively (Andersen and Plum, 1965; Plasse et al., 1968). Mean gestation length of Holstein hiefers bred to Angus bulls in the present study ($\overline{X} = 278.2$ days) was similar to that reported previously ($\overline{X} = 280.0$ days) by Boyd and Hafs (1965). Holstein heifers bred to Brahman bulls (285.0 days) had a longer gestation length than those bred to Holstein and Angus bulls (278.7 days). This is in agreement with previous research indicating that genotype of the conceptus contributes to variation in length of gestation (Plasse et al., 1968). Control of gestation length by the fetus was documented in a study of reciprocal embryo transfer between breeds of cattle eliciting long and short gestation length (King et al., 1982); length of gestation was not dependent upon breed of the recipient, but rather upon breed of the conceptus. The concept of initiation of parturition by the fetus that has been demonstrated in sheep (Liggins et al., 1973) is applicable, in part, to the cow in that maturation of the fetal pituitary-adrenal axis is responsible for

TABLE 6.7. Within-heifer partial and among-heifers gross correlation coefficient of responses in Holstein heifers.

	Within-heifer partial correlations[a]		
	Estradiol	Estrone sulfate	Progesterone
Estrone	0.68**	0.50**	-0.47**
Estradiol		0.58**	-0.44**
Estrone sulfate			-0.15*

	Among-heifers gross correlations[b]			
	Estrone	Estradiol	Estrone sulfate	PGFM
Calf birth weight	0.57**	0.59**	0.64**	0.56**

[a] Degrees of freedom = 286.
[b] Degrees of freedom = 19.
** P < 0.01; * P < 0.05.

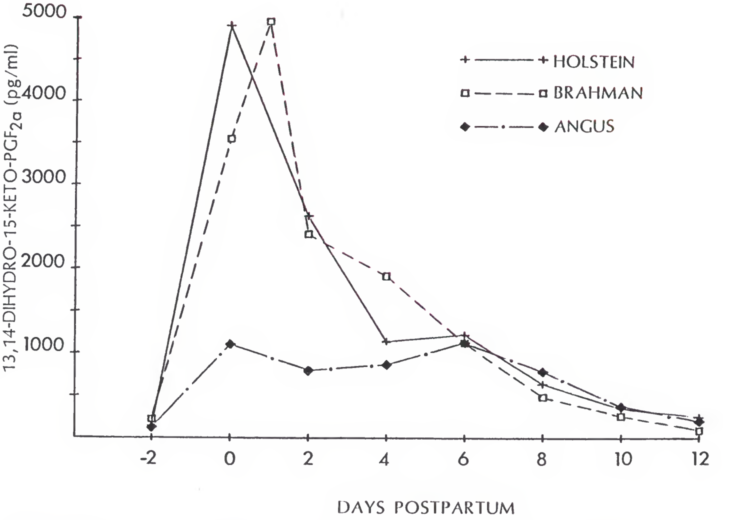


Figure 6.5 Profile of least squares daily means of 15-keto-13,14-dihydro-PGF$_{2\alpha}$ (PGFM) concentrations from day -2 postpartum to day 15 postpartum in Holstein heifers bred to either Holstein, Brahman or Angus bulls.

initiation of parturition. Prolonged gestation in cows has been associated with absence of adenohypophysis or fetal adrenals that were hypoplastic and unresponsive to ACTH (Holm, 1967; Kennedy, 1971). Furthermore, fetal plasma corticosteroids concentrations increased prior to parturition (Comline et al., 1974; Hunter et al., 1976) and infusion of corticotropin (Welch et al., 1973; Comline et al., 1974) or dexamethasone (Comline et al., 1974; Hunter et al., 1977) induced parturition in cows. Longer gestation length in the Brahman service sire group may be due to a slower maturation of the pituitary-adrenal axis in fetuses of this genotype.

Calf birth weights of Holstein heifers bred to Angus bulls were 15% lower than those of purebred Holstein heifers and agrees with research of Boyd and Hafs (1965). Mean birth weight of crossbred Holstein x Brahman calves was similar to that of purebred Holstein calves, but the variation in calf birth weight was larger for Holstein x Brahman calves (Table 6.2). Results of previous experiments involving reciprocal crosses between the large Shire horses and the small Shetland ponies (Walton and Hammond, 1938) and between large South Devon and small Dexter cattle (Joubert and Hammond, 1958) indicated that dams of larger breeds produced larger offspring. Such crossbreeding experiments between breeds reflect extreme variation in size of the parent and lead to the conclusion that "maternal regulation of fetal growth was very marked and obscured any genetic differences" (Walton and Hammond, 1938). Joubert and Hammond (1958) recognized that despite the importance of maternal regulation of fetal growth, the service sire could have considerable effects in determining calf birth weight. This was further substantiated in a large scale study

(Boyd and Hafs, 1965) where sire of fetus or breed of the sire of fetus significantly affected the birth weight of calves. Influence of the breed of the service sire on calf birth weight is given additional support in the present experiment. Furthermore, endocrine responses of the maternal units were altered by genotype of the conceptus they bore.

The gradual decline in progesterone concentrations until the last 2 to 3 days prepartum and the precipitous decrease thereafter agree with previous observations (Smith et al., 1973; Hoffmann et al., 1973; Eley et al., 1981a; Collier et al., 1982). Maintenance of higher prepartum concentrations of progesterone in the Brahman and Holstein than in the Angus service sire group-breed suggests that the conceptus may contribute to the regulation of progesterone synthesis and metabolism during pregnancy. In cattle, the corpus luteum appears to be the major source of progesterone during gestation (Thorburn et al., 1977) whereas contributions by the adrenal glands or the placenta are relatively minor (Schmidt et al., 1977; Hoffmann et al., 1979). Maintenance of normal luteal function and pregnancy after hypophyseal stalk transection of beef heifers in midpregnancy indicated that production of progesterone by the corpus luteum is not exclusively under the control of the maternal pituitary via its secretion of LH (Anderson et al., 1979). Recently, presence of a chorionic gonadotropin-like protein with luteotrophic activity was demonstrated in extracts of bovine cotyledons collected between 50 and 100 days of gestation (Ailenberg and Shemesh, 1983). They suggested that a luteotrophic factor of conceptus origin may regulate progesterone synthesis by the corpus luteum during pregnancy. Results of the present experiment provide further evidence that corpus luteum function may be under control of the conceptus. Differences in peripheral concentrations of progesterone between service sire-breed groups may reflect differential production of a chorionic gonadotropin-like protein by genetically different conceptus units; crossbred Angus x Holstein conceptuses would produce less placental luteotrophic hormone than crossbred Brahman x Holstein and purebred Holstein x Holstein conceptuses.

The prepartum increase in free and conjugated estrogens agrees with previous observations in cattle during late gestation (Robinson et al., 1971; Smith et al., 1973; Comline et al., 1974; Eley et al., 1981a; Collier et al., 1982). Higher concentrations of sulpho conjugated estrogens (estrone sulfate) and relatively higher concentrations of estrone than estradiol among the free estrogens measured are also in agreement with the findings of Tsang et al. (1975), Robertson and King (1979), Hoffmann et al. (1979), Eley et al. (1979) and Eley et al. (1981a). The bovine placenta is likely to be a major source of estrogen production, since it contains an active aromatase system (Ainsworth and Ryan, 1966) and utero-ovarian vein concentrations of unconjugated estrogens are greater than those in the jugular vein (Comline et al., 1974; Peterson et al., 1975) or the uterine artery (Ferrell and Ford, 1980; Ferrell et al., 1983). In vitro and in vivo experiments (Hoffmann et al., 1976; 1979) indicated that the bovine placentome is the major source of estrogens near term. Estrone and estradiol-17_β are the major estrogens produced and a significant portion is conjugated at the site of synthesis, in the fetal cotyledon. While estradiol-17_α sulfate is the major estrogen in the fetal circulation (Hoffmann et al., 1979), estrone sulfate predominates in the maternal circulation (Hoffmann et al., 1979; Robertson and King, 1979). Source of unconjugated estrogens in the maternal circulation is unclear. Concentrations of estrone, but not of estradiol, were higher in uterine venous than in jugular venous blood during late pregnancy in cows (Hoffman et al., 1976). Furthermore, Ferrell and Ford (1980) reported a net uterine production of estrone, but not of estradiol, from days 140 to 245 in cattle. Collectively, these results suggest that estrone is secreted by tissue within the gravid bovine uterus, possibly by the placentome whereas estradiol is not. The possibility exists that rises in estrone and estradiol close to parturition also may be due to increased sulfatase activity in the mammary gland. Walker and Peaker (1978) demonstrated that the goat mammary gland could account for over 90% of the rise in estradiol during the last two weeks of pregnancy. The source of unconjugated estrogens (estrone and estradiol) in dairy cows cannot be determined in the present experiment, but concentrations of estrone and estradiol were correlated highly with those of estrone sulfate (Table 6.7).

Concentrations of estrone, estradiol and estrone sulfate were different among groups of Holstein heifers bearing genetically different conceptuses. Results of the present experiment suggest that production of estrogen by the fetal placenta (cotyledon) was lower in the Angus service sire group (Figures 6.2, 6.3 and 6.4). Gestation length cannot account totally for higher concentrations of the three estrogens measured (estrone, estradiol and estrone sulfate) in the Holstein and Brahman service sire groups than in the Angus service sire group. Response curves of estrone, estradiol and estrone sulfate concentrations were lower appreciably in the Angus than in the Holstein service sire group although gestation lengths were similar in both groups. However, the possibility exists that, approaching parturition, the more rapid increase in estrogen concentrations in the Brahman service sire group was related to a longer gestation length in this group. Longer gestation length may favor an increased maturation of the placentome which in turn may be associated with a faster increase in estrogen concentrations, close to parturition, in the Brahman service sire group.

Alterations of estrogen concentrations, measured in the maternal circulation, were directly attributable to differences in breed of the service sire and would suggest independent control of estrogen concentrations by the conceptus itself. Peripheral concentrations of estrogens were affected in two groups of genetically different Jersey cows bearing conceptuses that were differentially selected for milk yield (Eley et al., 1981a). However, maternal and conceptus control of estrogen production could not be separated in their experimental model. Within service sire-breed groups, peripheral concentrations of estradiol and estrone sulfate which may reflect estrogenic production by the conceptus were the only responses that were affected significantly by the service sire (Table 6.4). This further exemplifies the effect of the service sire on the maternal unit via the conceptus she bore and is in agreement with results of Osinga (1978) who indicated that the sire or breed of the sire of fetus influenced the urinary estrogen/creatinine ratio during late pregnancy in cows. Large variability of estrone sulfate among Holstein heifers within group may reflect real differences between conceptus units that become even greater when fetal and maternal units are genetically different (Table 6.6). Greater antigenic dissimilarity was related to larger placentas in mice (James, 1967) and higher calf birth weight in cows (Ousted, 1978).

The small number of heifers in each service sire group limited proper evaluation of estimates of milk yield (Appendix C; Table C.7) and no statistical differences were detected between service sire-breed group, but there was a tendency for milk and fat yield to be lower in the Brahman service sire group. Present results provide additional support to the concept that variation in secretion of estrogen and possibly bovine placental lactogen by the fetus contributes to the mechanism by which sire of fetus exerts its influences on subsequent milk and fat yield of the dam after calving (Adkinson et al., 1977; Thatcher et al., 1980).

Release of estrogen of conceptus origin into the maternal circulation may be associated with specific factors or responses of maternal and fetal units (Thatcher et al., 1980) that ultimately contribute to variation in calf birth weight. Calf birth weight (Collier et al., 1980) and bovine placental weight (Head et al., 1981) were lower during the hot summer months. A positive relationship (r = 0.84) between total concentrations of estrogens at 220 days of gestation and calf birth weight was detected (Terqui et al., 1975). Furthermore, lower concentrations of prepartum estrone sulfate were associated with lower calf birth weights in Holstein cows that were heat stressed during the last trimester of gestation (Collier et al., 1982). Early in pregnancy, the placenta is appreciably larger than the fetus. However, during later stages of development in sheep

(Alexander, 1964) and cows (Eley et al., 1978), absolute growth of the fetus far exceeds that of the placenta. Transfer of respiratory gases and nutrients between maternal and fetal circulations constitutes one of the more important functions of the placenta. Functional properties of the placenta change during gestation. During the last trimester of pregnancy, maturation of the sheep placenta is characterized by substantial increase in the function of the placenta as an organ of exchange between the mother and the fetus (Kulhanek et al., 1974; Longo and Ching, 1977). Permeability to urea at 90 days in the sheep placenta was only 20% of that measured in term placenta (Kulhanek et al., 1974); furthermore, placental diffusing capacity to carbon monoxide (Longo and Ching, 1977) and to oxygen (Battaglia et al., 1965) increased as a function of gestational age. Attempts have been made, mainly in human medicine, to relate measurements of urinary or plasma estrogens with feto-placental function. An association could be established between low estrogens and compromised fetal wellbeing (Jorgensen and Frandsen, 1973). In the present experiment, lower concentrations of estrogens during the last phase of pregnancy in the Angus service sire group was associated with a lower calf birth weight. Furthermore, among the 21 heifers, a positive correlation was detected between calf birth weight and least squares means for concentrations of estrone, estradiol and estrone sulfate (Table 6.7). Since concentrations of maternal estrogens were related to conceptus genotype as reflected by differential concentrations of estrogens among the various service sire-breed groups (Figures 6.2, 6.3 and 6.4), results suggest that measurement of maternal concentrations of estrogens is a functional index of the fetal part of the placentome. This estrogenic response may represent the capacity of the conceptus to regulate its intrauterine environment to achieve growth at a rate either imposed by its genetic code or that is compatible with the environment provided by the maternal unit (Thatcher et al., 1980). Considerably higher concentrations of estrone sulfate compared to the concentrations of unconjugated estrone or estradiol in cattle would favor estrone sulfate as the estrogen of choice to assess placental function when measured in the maternal circulation of cattle.

Although precursors for estrogen synthesis by the bovine cotyledon is unknown, the negative correlation (Table 6.7) between concentrations of progesterone and the three estrogens (estrone, estradiol, and estrone sulfate) suggest that progesterone from the corpus luteum may be metabolized into estrogens by the bovine placentome (cotyledon and caruncle). Hoffmann et al. (1979) indicated that precursors for estrogen synthesis in the bovine fetal cotyledon must be supplied by the maternal circulation. An uptake of progesterone by the gravid uterus was reported previously (Comline et al., 1974; Wagner et al., 1974; Hoffmann et al., 1976; Ferrell et al., 1983). Maintenance of low progesterone concentrations in umbilical venous blood, despite its uptake from maternal uterine arterial blood (Ferrell et al., 1983), suggest that progesterone may be metabolized by the bovine placenta instead of being redirected integrally toward the fetal circulation. Conversion of C_{21} steroids (17_{α}-hydroxy progesterone and pregnenolone) to estrogens by the bovine cotyledon was reported (Evans and Wagner, 1981). A lower profile of both prepartum progesterone and estrogen concentrations in the Angus service sire-breed group may indicate that dynamics of estrogen synthesis are dependent upon availability of substrate (progesterone) to the cotyledon. Therefore, control of estrogen production may be exerted indirectly by modulation of substrate availability via a gonadotropin-like luteotrophin of conceptus origin (Ailenberg and Shemesh, 1983). This would constitute a critical mechanism governing feto-placental growth owing to the influence of steroids on development and maturation of the fetus during intrauterine life (Jost, 1969; 1970; Gorski, 1971). Likewise several factors implicated in the process of parturition appear to be dependent upon an increase in estrogen production which is stimulated by fetal adrenal cortisol (Comline et al, 1974; Hunter et al., 1977; Flint et al., 1979). Synthesis of oxytocin receptors in the guinea pig (Alexandrova and Soloff, 1980) and the stimulus for prostaglandin production in goats (Currie and Thorburn, 1977) appear to arise from an increase in estrogen production. Furthermore, Garfield et al. (1980) proposed that steroid hormones regulate synthesis of myometrial gap junctions via an action of prostaglandins on gap junction assembly. Differential increase in estrogen synthesis may reflect an active role of the conceptus in preparing the dam for parturition. It would be futile for the conceptus, which initiates the process of parturition (Holm, 1967; Kennedy, 1971; Thorburn et al., 1977), not to condition the dam in order to prepare optimal conditions for its own delivery.

Postpartum profiles of PGFM concentration in the three groups of service sire-breed were similar to those reported earlier (Edqvist et al., 1978; Eley et al., 1981b; Lindell et al., 1982; Chapters III and V). Concentrations of PGFM increased at parturition, peaked at 1 to 2 days postpartum, and remained elevated during 10 to 15 days postpartum. In Chapter III, the uterus has been identified as the main source of F series prostaglandins during the early postpartum period. In addition, the postpartum uterus may contribute to the peripheral concentrations of PGFM by synthesis of $PGF_{2\alpha}$ and its metabolism in uterine caruncular tissue (Chapter III). A lower postpartum profile of PGFM concentrations in the Angus service sire group reflected prepartum differences among service sire-breed groups (estrone, estradiol and estrone sulfate were lower in Angus service sire group) and was associated also with a lower calf birth weight. These differences in postpartum PGFM may represent an environmental effect of the conceptus on the maternal unit (caruncle) exerted, during gestation, in some manner other than by direct transmission of genes. However, this conceptus-induced environmental effect is not expressed until the postpartum period.

In ruminants, caruncular development is stimulated by the physical presence of the conceptus (Amoroso, 1952; Alexander, 1964). Sir John Hammond (1961) defined the last stage of conceptus development as the fetal stage, in which nutrition is considered to be accomplished "by assisted transfusion between maternal and fetal blood streams in the placentome". Apparently, this would be achieved by increasing placental villous surface area and vascularity (Alexander, 1978). Barcroft and Barron (1946) reported increasing vascularity of the maternal part of the placentome even when its weight was declining toward the end of gestation in sheep. Furthermore, increasing development of the caruncular vasculature occurred between 241 and 273 days of gestation in the cow (Hutchinson, 1962). Several lines of evidences suggest that placental vascularization is not a passive process, but is rather dependent upon the environmental conditions provided by the mother to the fetus or upon fetal stimulation of the maternal unit. Such fetal and maternal interactions are evident from experiments in which placental development was altered by either partial ligation of blood supply to the placenta (Emmanouilides et al., 1968; Hobel et al., 1970), embolization of the uteroplacental circulation with microspheres (Clapp et al., 1981), caruncIectomy (Alexander, 1964; Robinson et al., 1979), hemihysterectomy or ligation of one uterine horn of the bipartite sheep uterus (Cefalo et al., 1977; Caton et al., 1983) or oxygen deprivation during the course of pregnancy (Metcalfe, 1970; Metcalfe et al., 1977; Gilbert et al., 1979). Although achievement of normal growth of the fetus and birth weight were dependent upon the nature of the insult, in all instances compensatory growth occurred in the fetal and/or in the maternal part of the placentome. These gross morphological changes, which reflected to a degree fetal and maternal interactions, were expressed microscopically by changes in surface area of the placenta (Smith et al., 1983) or in maternal and fetal placental vascularization (Van der Heijden, 1981).

In the present experiment, genetically different conceptus groups caused a differential stimulation of the maternal unit which in turn responded by providing a sufficient intrauterine environment (caruncles) that permitted ultimate growth of the fetus to its programmed genetic size. It has been shown in Chapter IV that postpartum peripheral concentrations of PGFM are a reliable indicator of local uterine production of F series prostaglandins. It was also suggested that peripheral PGFM concentrations measured postpartum may reflect the degree of caruncular growth and/or vascularization in response to feto-maternal interactions during gestation. The lower concentrations of PGFM in the Angus service sire group may reflect a lower degree of caruncular development and/or vascularization that was imposed on the maternal unit by the conceptus genotype during pregnancy to meet its needs.

Cows showing clinical signs of postpartum uterine disorder had a prolonged release of PGFM in peripheral circulation (Lindell et al., 1982). Therefore, the possibility exists that postpartum uterine release of F series prostaglandins, as reflected by peripheral PGFM concentrations, may have been altered in heifers that experienced parturient and postpartum clinical disturbances (e.g., dystocia, calf mortality, uterine infection). Analysis of the profile of PGFM concentrations for the first 12 days postpartum in heifers with and without clinical problem did not reveal differences between these two groups of animals. Furthermore, parturient and postpartum clinical disturbances occurred at approximately the same frequency in each service sire-breed group (Appendix C; Table C.1). Thus, clinical problems cannot account for the difference in postpartum profile of PGFM observed between service sire-breed groups. In the present study, magnitude of the PGFM concentration early postpartum was the major factor differentiating the various service sire-breed groups. Caruncles become necrotic within the first 5 days postpartum and all of the caruncular mass involved in the placentome is sloughed by day 15 (Marion and Gier, 1968). Study of the magnitude of PGFM concentrations early postpartum may be related in part to the caruncular mass, whereas study of duration of the prostaglandin release may deal with uterine tissue repair as suggested by Lindell et al. (1982).

It is unlikely that higher postpartum concentrations of PGFM in the Holstein and Brahman service sire groups was due simply to a larger calf birth weight. In Holstein cows heat stressed during the last trimester of gestation, higher concentrations of PGFM were measured despite a lower calf birth weight (Lewis et al., 1984). It was suggested in the latter study, that higher postpartum concentrations of PGFM may be related to an increase in vascularization of the caruncles to compensate for a decrease in uterine blood flow of cows heat stressed during the last trimester of pregnancy.

Genetic (present study) and prepartum environmental manipulations of the conceptus (Lewis et al., 1984) lead to alteration of the postpartum concentrations of PGFM that may be related to an earlier response of the maternal unit to maintain a proper environment for conceptus development. Collectively, these results support the hypothesis, previously stated (Chapter IV), that measurements of postpartum peripheral concentrations of PGFM may be a functional index of the maternal part of the placentome. In addition, results of the present study also suggest that prepartum measurements of estrogens, mainly estrone sulfate, may be a functional index of the fetal part of the placentome. It is clear from this designed experiment that fetal genotype (primarily due to sire of fetus) influences various endocrine and physiological responses during the periparturient period.

CHAPTER VII
CARRY-OVER EFFECTS OF PERIPARTURIENT ENDOCRINE CHANGES ON POSTPARTUM REPRODUCTIVE FUNCTION OF HOLSTEIN HEIFERS BRED TO GENETICALLY DIFFERENT SERVICE SIRES

Introduction

Reestablishment of a functional hypothalamo-pituitary-ovarian axis, ovarian follicular growth (Stevenson and Britt, 1980) and uterine involution (Gier and Marion, 1968) are processes involved in restoration of postpartum reproductive efficiency in cattle. The complex series of hormonal changes characterizing the periparturient period in cattle may influence the processes leading to resumption of postpartum reproductive function (Thatcher et al., 1980; Eley et al., 1981b). Results presented in the preceeding chapter demonstrated that genotype of the conceptus altered the periparturient endocrine balance of the maternal unit (Holstein heifers). Therefore, the objective of this experiment was to evaluate the potential carry-over effect of conceptus-induced changes of the maternal unit on postpartum reproductive performance of Holstein heifers bred to either Holstein, Brahman or Angus bulls.

Materials and Methods

Design

Animals used, experimental design and bleeding schedule have been presented in Chapter VI. Briefly, 21 virgin Holstein heifers were assigned to three service sire-breed groups in which heifers were inseminated artificially to either purebred Holstein (n = 7), Brahman (n = 7), or Angus bulls (n = 7). With this type of experimental design, measurements of maternal responses provided a sensitive estimate of conceptus genotype due primarily to breed of the service sire. Blood samples were collected from day -160 prepartum to day 60 postpartum and plasma obtained by centrifugation. Concentrations of progesterone, estrone, estradiol and estrone sulphate from day -21 prepartum until parturition (day 0) and of 15-keto-13,14-dihydro-$PGF_{2\alpha}$ (PGFM) from day -2 prepartum to day 15 postpartum were presented in Chapter VI.

Rectal Palpation

The reproductive tract of each cow was examined rectally, three times a week, from days 5 to 60 postpartum. Examination was performed immediately after blood samples had been collected. Measurements made on reproductive organs by palpation were: cervical diameter, diameter of each uterine horn at the external bifurcation, and presence or absence of follicles and/or corpus luteum on each ovary. The gravid uterine horn was identified from records of previous pregnancy determination made rectally between days 45 to 60 of gestation and confirmed by rectal palpation of the larger uterine horn after parturition. Measurements of uterine horn diameters and determination of presence or absence of ovarian structures were recorded in relation to the previously gravid uterine horn.

Pituitary responsiveness

To evaluate pituitary responsiveness of Holstein heifers bred to genetically different service sires, simultaneous injections of thyrotropin releasing hormone (TRH; Sigma Chemical Co., St. Louis, MO) and gonadotropin releasing hormone (GnRH; Sigma Chemical Co., St. Louis, MO) were administered to each animal on day 10 postpartum. On the day prior to injection, an indwelling polyvinyl catheter (0.86 mm id x 1.52 mm od; V-6, Bolab Inc., Derry, NH) was inserted into the jugular vein. Two milligrams of TRH were dissolved into 40 ml of sterile 0.9% physiological saline to give a concentration of 100 ug/2 ml. A comparable solution was prepared with GnRH. Two milliliters of each solution were pooled, and 4 ml of a mixed solution of GnRH and TRH (GnRH-TRH) were injected into the jugular catheter. Following injection, catheter was flushed with 5 ml of heparinized saline (40 i.u./ml).

During the 1 h preceeding the intravenous injection of GnRH-TRH, blood samples (5 ml) were collected into heparinized syringes at 20 min intervals. After the injection, blood samples were withdrawn at 2, 5, 10, 20, 30, 40, 60, 70, 80 and 90 min intervals followed by 30 min sampling intervals until 6 h postinjection. Upon collection, samples were transferred into heparinized tubes (100 i.u./tube) and placed immediately into an ice bath. Plasma was obtained by centrifugation (5000 x g) at 4 C and stored at -20 C until assayed for LH concentrations as described below.

Assay Procedures

Progesterone concentrations were measured in plasma samples collected between parturition (day 0) and day 60 postpartum. A description of the assay procedure has been reported previously (Eley et al., 1981a) and summarized in Chapter V. Samples were assayed in duplicate by extracting 100 and 200 ul of plasma for determination of postpartum concentrations of progesterone. Postpartum samples were analyzed in two large assays. Twelve repeated measurements of a reference sample resulted in intra- and interassay coefficients of variation of 8.38 and 7.86%, respectively.

Concentrations of LH were measured in experimental plasma samples collected from days 1 to 10 postpartum and those collected during the 1 h preceeding and the 6 h following injection of GnRH-TRH. The method used was a modification of the procedure described by Niswender et al. (1969). Description and validation of the assay procedure have been given in detail in Chapter V. Duplicate determinations of basal concentrations of LH from days 1 to 10 postpartum were made with plasma volumes of 200 and 300 ul.

Determination of LH response to GnRH-TRH challenge was measured in triplicate utilizing plasma volumes of 100, 200 and 300 ul. Repeated measurements of reference plasma samples gave intra- and interassay coefficients of variation of 16.7% and 19.7%, respectively. Prolactin responses to TRH injections will not be presented in this chapter.

Statistical Analysis

Least squares analyses of variance of postpartum hormonal responses and of measurements of cervical and uterine horn diameter were conducted utilizing the General Linear Models procedure of the Statistical Analysis System (SAS; Barr et al., 1979). Experimental design was in the nature of a split split plot in which service sires were nested in breed (of service sire) and heifers were nested in service sire and in breed (see Chapter VI). Repeated measurements over time were taken in heifers, and time (day or minute) was considered a continuous independent variable. Each response was characterized by a time trend which was analyzed by polynomial regression. Tests of homogeneity of regression were used to detect differences in time trends for postpartum responses between service sire-breed groups. In addition, when response curves of service sire-breed groups were not parallel ($P < 0.05$), orthogonal contrasts of response curves for service sire-breed groups were made as follows: Holstein, Brahman versus Angus; Holstein versus Brahman.

Agreement between rise in plasma progesterone concentrations and detection of palpable CL was poor ($r = 0.25$) in the present study. For this reason, analysis of ovarian activity (presence or absence of CL and/or follicle) as described in Chapter V was not performed in the present study. A change in ovarian morphology following a GnRH-TRH injection on day 10 postpartum in heifers, may have contributed to the low association between presence of a CL, determined by rectal palpation, with increases in progesterone concentrations in plasma.

Two different approaches were taken to analyze plasma progesterone concentrations postpartum. Initially, timing and duration of the first rise in progesterone, in response to a GnRH-TRH injection administered on day 10 postpartum, were analyzed individually for each heifer within service sire-breed group. In a second approach, values of progesterone concentrations for each heifer within service sire-breed group were accumulated additively, as described previously in Chapter V. This was done to characterize overall progesterone response (accumulated progesterone) over the first 60 days postpartum in each service sire-breed group. Least squares analysis and statistical model used to analyze accumulated values of progesterone concentrations were identical to those described in the present chapter for analysis of LH concentrations and cervical diameter. Due to the high sensitivity of the statistical test using the transformed values of progesterone concentrations (accumulated progesterone), level of probability of less than 0.01 was arbitrarily required to indicate that profiles of accumulated progesterone differ between service sire-breed groups.

Palpable changes in uterine horn diameter were analyzed according to the side on which measurements were made relative to the previously gravid uterine horn (e.g., uterine horn: previously gravid versus nongravid side). The mathematical model includes source of variation due to service sire-breed groups (breed), service sire within breed, cow within service sire and within breed, side, and interactions of breed, service sire within breed and cow within service sire and within breed with side (see Table 7.2). Day was considered a continuous independent variable, and tests of homogeneity of regression were used to detect differences in response curves between service sire-breed groups, side and the interaction of service sire-breed group and side. A caesarian section was performed on one heifer assigned to the Brahman service sire group (see Chapter VI). This heifer was not palpated postpartum.

Clinical difficulties at parturition and early postpartum slow the processes of cervical and uterine involution (Fonseca et al., 1983). In the present experiment, such problems occurred (see Chapter VI and Appendix C; Table C.1) which may have confounded effects of breed of the service sire on rates of cervical and uterine involution. However, since clinical difficulties during parturition or the postpartum period occurred at a similar frequency in each service sire-breed group (e.g., Holstein = 3/7; Brahman = 3/6; Angus = 3/7; see Appendix C; Table C.1), differences observed in rates of cervical and uterine involution were interpreted as being due to breed of the service sire and not only to clinical difficulties.

Results

Mean plasma concentrations of LH did not differ between service sire-breed groups during the first 10 days postpartum ($P > 0.1$; Tables 7.1 and 7.2). Likewise, there was no evidence that increases in LH concentrations from days 1 to 10 postpartum were different between service sire-breed groups ($P > 0.1$; Table 7.3). Average concentrations of LH were 0.13 ng/ml on day 1 and increased to 0.40 ng/ml on day 10 postpartum.

Overall mean concentrations of LH measured in plasma samples collected before or after a GnRH-TRH injection were not different between service sire-breed groups ($P > 0.1$; Tables 7.1 and 7.2). Similarly, increases in profile of plasma concentrations of LH released after GnRH-TRH injection did not differ between service sire-breed groups ($P > 0.1$; Table 7.3). The pooled treatment response curve for plasma LH concentrations after GnRH-TRH injection is depicted in Figure 7.1. Mean concentrations of LH were 0.73 ng/ml at the time of injection and at 60 min postinjection obtained a peak of 4.30 ng/ml.

For each heifer, timing and duration of the first rise in plasma progesterone concentrations after a GnRH-TRH injection, administered on day 10 postpartum, are presented in Table 7.5. Two heifers in the Holstein service sire group had a short duration increase in progesterone concentrations above 1 ng/ml prior to the day of GnRH-TRH

TABLE 7.1. Least squares means of postpartum responses in Holstein heifers bred to either Holstein, Brahman or Angus bulls.

	Service sire-breed group			
	Holstein	Brahman	Angus	S.E.M.[a]
LH (days 1 to 10) (ng/ml)	0.28	0.32	0.30	±0.09
LH (GnRH-TRH challenge) pre-injection (ng/ml)	0.67	0.87	0.64	±0.24
post-injection (ng/ml)	2.48	2.44	1.68	±0.68
progesterone (day 1 to 60) (pg/ml)	1609	1626	1678	±378
cervical diameter (mm)	45.9[b]	46.0[b]	41.0[c]	±1.56
uterine horn diameter[d]	39.3	38.9	35.3	±1.25

[a] S.E.M. = standard errors of the mean; they are approximate, conservative and similar for each group.

[b,c] Values in a row with different superscript are significantly different ($P < 0.05$).

[d] Previously gravid uterine horn.

TABLE 7.2. Least squares analyses of variance of postpartum responses in Holstein heifers bred to either Holstein, Brahman or Angus bulls.

Source of Variation	LH		LH(GnRH)		Accumulated progesterone		Cervical diameter		
	df	MS	df	MS	df	MS[a]	df	MS	Error term
Breed	2	0.05	2	21.32	2	5583	2	1398*	Service sire (breed)
Service sire (breed)	9	0.63	9	67.48	9	27778	8	202	Heifer (service sire, breed)
Heifer (service sire, breed)	9	0.55**	9	86.88**	9	15186*	9	396**	Residual
Times[b,c]	1	2.60**	5	89.34**	2	1155800*	4	16773**	Residual
Residual	189	0.07	475	2.27	718	818	427	52	

Source of variation	Uterine horn diameter		Error term
	df	MS	
Breed	2	625	Service sire (breed)
Service sire (breed)	8	198	Heifer (service sire,breed)
Heifer (service sire,breed)	9	179**	Residual
Side	1	13840**	Residual
Breed x side	2	105	Service sire (breed) x side
Service sire (breed) x side	8	60*	Heifer (service sire,breed) x side
Heifer (service sire,breed) x side	9	14	Residual
Days[b]	4	10432**	Residual
Residual	803	37	

[a] MS = MS x 10^5; MS = mean square, df = degrees of freedom.

[b] See Table 7.3 for test of homogeneity of regression.

[c] Times = days for LH, accumulated progesterone and cervical diameter responses; times = minutes for LH(GnRH) response.

** $P < 0.01$; * $P < 0.05$.

TABLE 7.3. Test of homogeneity of regression and orthogonal contrast of postpartum responses in Holstein heifers bred to either Holstein, Brahman or Angus bulls.

Response	Order of regression	Error term		Treatment		Orthogonal[c] contrast	
		df[a]	MS[a]	df[b]	MS_D[b]	A	B
LH (day 1 to 10)	1	187	0.070	2	0.005	NS	NS
LH (GnRH-TRH challenge)	5	465	2.27	10	2.43	NS	NS
Accumulated progesterone (days 1 to 60)	2	714	80973861	4	242253242*	**	NS
Cervical diameter	4	419	40.66	8	662.00**	**	NS

[a] Degrees of freedom (df) and mean square (MS_R) associated with the error term when individual curves were generated for each treatment.

[b] Degrees of freedom (df) and mean square (MS_D) associated with the difference in residual sums of squares between fitting one pooled curve versus an individual curve for each treatment.

[c] Orthogonal contrast: A = Holstein, Brahman versus Angus
B = Holstein versus Brahman

** $P < 0.01$; * $P < 0.05$.

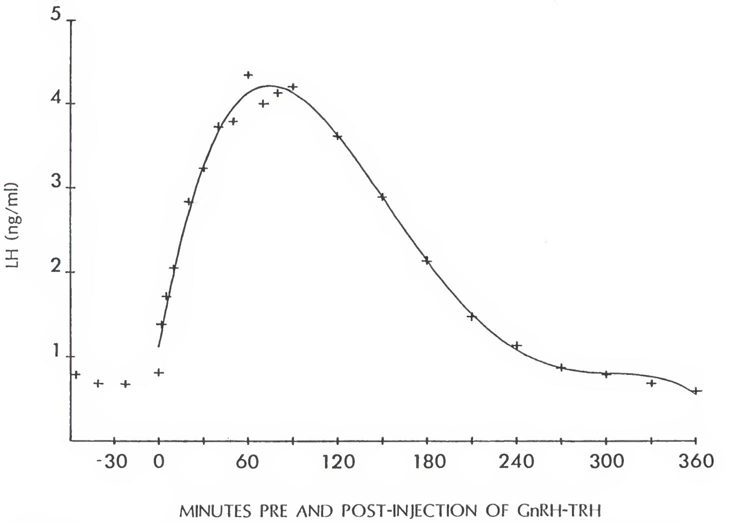


Figure 7.1 Pooled least squares regression and least squares means of luteinizing hormone (LH) response to GnRH-TRH administration on day 10 postpartum for Holstein heifers bred to either Holstein, Brahman or Angus bulls.

injection. One heifer in the Brahman service sire group never had an increase in progesterone concentrations above 1 ng/ml within the first 60 days postpartum and was not included in calculations of timing or duration of progesterone rise. There were no statistical differences ($P > 0.1$; Table 7.5) between service sire-breed groups in mean timing or duration of the first progesterone rise. However, the first increase in progesterone concentration was better synchronized (smaller standard error of the mean) in the Angus service sire group. All heifers in the Angus service sire group had a progesterone rise, within 9 days after the GnRH-TRH injection (range = 13-19 days), whereas range of days for progesterone to increase above 1 ng/ml were 13 to 29 and 15 to 29 for heifers in the Holstein and Brahman service sire groups, respectively.

There was no difference between service sire-breed groups in mean progesterone concentrations during the 60 days postpartum (Table 7.1). Profile of accumulated progesterone over the first 50 days postpartum tended to be different between the three service sire-breed groups ($P < 0.05$; Table 7.3). Progesterone accumulated faster in the Angus service sire group than in the Holstein and the Brahman service sire group ($P < 0.01$; Table 7.3; Figure 7.2). However, there was no difference in profile of accumulated progesterone concentrations between the Holstein and the Brahman service sire groups. The faster rate of accumulated progesterone early postpartum in the Angus service sire group (Figure 7.2) most probably reflects the tendency for heifers of this group to respond earlier (within 9 days) to the GnRH-TRH injection given on day 10 postpartum (Table 7.5).

TABLE 7.4. Test of homogeneity of regression for postpartum changes in previously gravid and nongravid (Side) uterine horn diameters in Holstein heifers bred to either Holstein, Brahman or Angus bulls.

		Error term		Treatment		Side		Treatment x Side	
	Order of regression	df[a]	MS[a]	df[b]	MS$_D$[b]	dF[b]	MS$_D$[b]	df[b]	MS$_D$[b]
Uterine horn diameters	4	783	23.49	8	305.25**	4	1919**	8	57.75

[a] Degrees of freedom (df) and mean square (MS) associated with the error term when individual curves are generated for each treatment and each side.

[b] Degrees of freedom (df) and mean square (MS$_D$) associated with the difference in residual sums of squares between fitting one pooled curve versus an individual curve for each treatment, an individual curve for each side (ipsilateral or contralateral) or an individual curve for each side within each treatment.

** $P < 0.01$.

TABLE 7.5. Timing (day) and duration (days) of first rise in progesterone concentrations (progesterone > 1.0 ng/ml) occurring after an injection of GnRH-TRH (100 ug) given on day 10 postpartum in Holstein heifers bred to either Holstein, Brahman or Angus bulls.

Observation number	Service sire-breed group					
	Holstein		Brahman		Angus	
	timing	duration	timing	duration	timing	duration
1	29	4	60	-	19	7
2	23	3	29	27	19	1
3	16	1	29	10	18	8
4	15[a]	12	23	10	17	2
5	15	5	20	4	15	21
6	14[a]	14	15	11	14	12
7	13	7	15	5	13	10
$\overline{X}$ ± S.E.M.[b]	17.8±2.2	6.57±1.8	21.8±2.6[c]	11.2±3.4[c]	16.4±0.9	8.7±2.5

[a] Prior to GnRH-TRH injection, a progesterone rise was detected on day 5 (observation no. 4) and on day 9 (observation no. 6) which lasted 3 and 1 days, respectively.

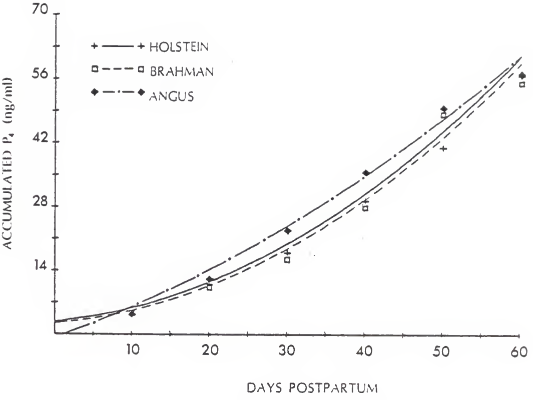
[b] Mean ± standard error of the mean.

[c] Observation number 1 not included.

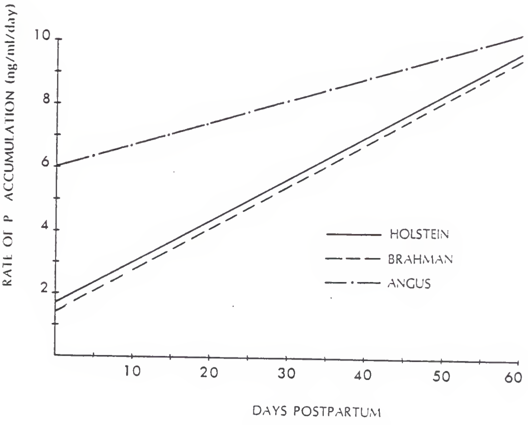
Least squares analysis of variance of postpartum changes in cervical and uterine size are presented in Table 7.2. Mean cervical diameter during the first 60 days postpartum differed ($P < 0.05$; Table 7.1) between service sire-breed groups. Mean cervical diameter was 41.0 mm in the Angus service sire group and was lower ($P < 0.05$) than in the Holstein and the Brahman service sire groups (45.9 and 46.0 mm, respectively; S.E.M. = ± 1.56). Although mean diameter of the previously gravid uterine horn tended to differ in the same direction than the cervical diameter, these differences were not statistically significant ($P > 0.1$; Table 7.2). Mean uterine horn diameters of the previously gravid uterine horn were 39.3, 38.9 and 35.3 mm in the Holstein, Brahman and Angus service sire group, respectively.

Polynomial regression equations that best described profiles of postpartum changes in cervical and uterine horn diameters are given in Appendix C (Table C.6). Day trends and tests of homogeneity of regression are presented in Tables 7.2, 7.3 and 7.4. Day trends of changes in cervical diameter were different between service sire-breed groups ($P< 0.01$; Table 7.3; Figure 7.3). Cervical diameter decreased in all groups, but was smaller in the Angus service sire group than in the Holstein and the Brahman service sire groups until day 32 postpartum when they became similar. For each service sire-breed group, first derivative of polynomial equations that described day trends of cervical diameter was calculated to obtain estimations of daily rates of reduction in cervical diameters. During the first 15 days postpartum, daily rate of reduction in cervical diameter was approximately 25% lower in the Angus service sire group than in the Holstein and

Figure 7.2 Least squares regressions and least squares means (every tenth day) of accumulated progesterone concentrations (upper panel) and rate of accumulation of progesterone (lower panel) from days 1 to 60 postpartum in Holstein heifers bred to either Holstein, Brahman or Angus bulls.

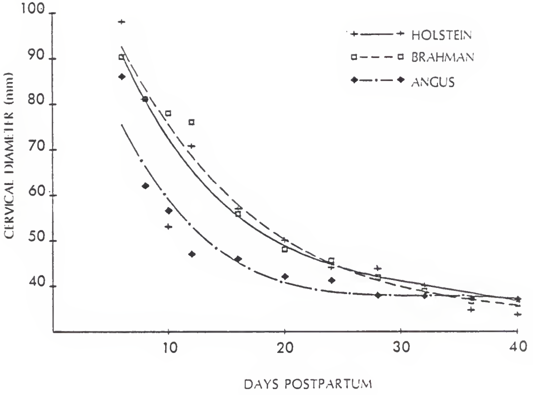


70
56
42
28
14
ACCUMULATED P4 (ng/ml)
HOLSTEIN
BRAHMAN
ANGUS
10
20
30
40
50
60
DAYS POSTPARTUM

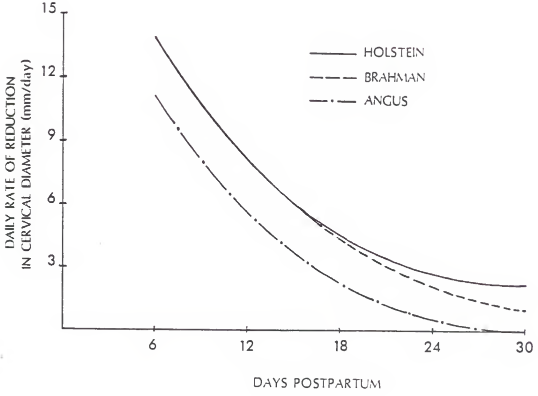


10
8
6
4
2
RATE OF P ACCUMULATION (ng/ml/day)
HOLSTEIN
BRAHMAN
ANGUS
10
20
30
40
50
60
DAYS POSTPARTUM

Figure 7.3 Least squares regressions and least squares means (every fourth day) of cervical diameter (upper panel) and of daily rate of reduction in cervical diameter (lower panel) from days 6 to 40 postpartum in Holstein heifers bred to either Holstein, Brahman or Angus bulls.



100
90
80
70
60
50
40
CERVICAL DIAMETER (mm)
10
20
30
40
DAYS POSTPARTUM
+——+ HOLSTEIN
□- - -□ BRAHMAN
◆—·—◆ ANGUS

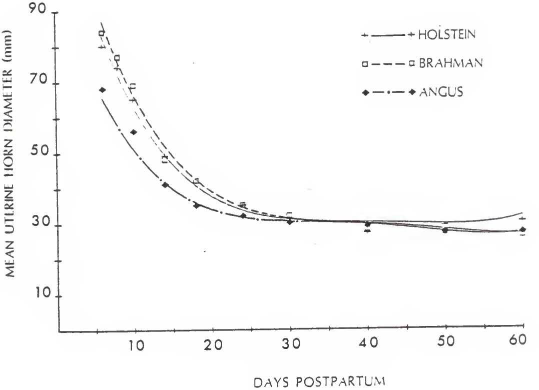


15
12
9
6
3
DAILY RATE OF REDUCTION IN CERVICAL DIAMETER (mm/day)
6
12
18
24
30
DAYS POSTPARTUM
—— HOLSTEIN
- - - BRAHMAN
—·— ANGUS

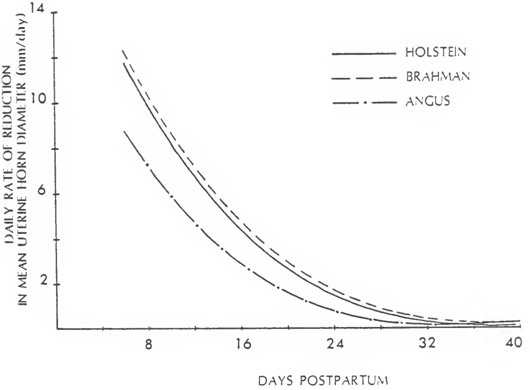
Brahman service sire groups (Figure 7.3). However, as the postpartum period extended, differences in cervical diameter were progressively smaller. By day 30, daily rate of reduction in cervical diameter was less than 2.5 mm in the three service sire-breed groups.

Least squares analysis of variance of changes in diameters of uterine horns are presented in Table 7.2. Pattern of change in reduction of uterine horn diameter was different between service sire-breed groups and between the previously gravid and nongravid uterine horns ($P < 0.01$; Table 7.4). Since there was no interaction ($P > 0.1$; Table 7.4) between service sire-breed groups and side on which diameter of uterine horn was measured, data illustrated in Figure 7.4 represented changes in mean uterine horn diameter (previously gravid + nongravid horn diameter divided by 2) during the first 60 days postpartum. From the first day that measurements of uterine horn diameter were made (day 5 postpartum) until day 32 postpartum, profile of reduction in mean uterine horn diameter was lower in the Angus service sire group than in the Holstein and Brahman service sire groups (Figure 7.4). Thereafter, size of uteri became similar in the three service sire-breed groups. However, daily rate of reduction in mean uterine horn diameter (Figure 7.4) was similar in the Holstein and Brahman service sire groups and appreciably higher than in the Angus service sire group. By day 8 postpartum, estimates of daily reduction in mean uterine horn diameter were approximately 33% lower in the Angus service sire group than in the average of the Holstein and Brahman service sire groups (8 versus 12 mm/day, respectively). The difference in daily rate of reduction in uterine horn diameter vanished progressively as the animals advanced into the

Figure 7.4 Least squares regressions and least squares means (every fourth day) of mean uterine horn diameters (upper panel) and of daily rate of reduction in mean uterine horn diameters (lower panel) from days 6 to 60 postpartum in Holstein heifers bred to either Holstein, Brahman or Angus bulls.



90
70
50
30
10
MEAN UTERINE HORN DIAMETER (mm)
10
20
30
40
50
60
DAYS POSTPARTUM
HOLSTEIN
BRAHMAN
ANGUS



14
10
6
2
DAILY RATE OF REDUCTION
IN MEAN UTERINE HORN DIAMETER (mm/day)
8
16
24
32
40
DAYS POSTPARTUM
HOLSTEIN
BRAHMAN
ANGUS

postpartum period. By day 32 postpartum, size of the uterus did not reduce farther in any group. Similar trends were observed when only changes in diameter of the previously gravid uterine horn were analyzed. On a within cow basis, significant ($P < 0.01$) positive correlations were detected between postpartum PGFM concentration (see Chapter VI) and cervical diameter ($r = 0.36$) and uterine horn diameter ($r = 0.32$).

Discussion

Results of the present experiment indicate that, within the first 10 days postpartum, pituitary secretion of LH was not altered in Holstein heifers that bore conceptuses of different genotype. Mean LH concentrations prior to day 10 postpartum, gradual increases in LH concentrations early postpartum, and LH responses to a GnRH-TRH injection were not different between service sire-breed groups. The low concentrations of LH measured on the day after parturition and their gradual increases thereafter are in agreement with previous observations in dairy cows (Echternkamp and Hansel, 1973; Kesler et al., 1977; Schams et al., 1978; Goodale et al., 1978; Webb et al., 1980; Eley et al., 1981a). Pulsatile patterns of LH secretion from the pituitary have been reported during the estrous cycle (Rahe et al., 1980) and the postpartum period in cows (Goodale et al., 1978; Carruthers and Hafs, 1980; Peters et al., 1981; Schallenberger and Peterson, 1982). In the present experiment, LH pulses could not be detected due to the infrequency of sampling. However, increases in amplitude and frequency of LH pulses during the early postpartum period in dairy cows, have been associated with an increase in basal plasma LH concentrations and reflected a gradual restoration of pituitary function (Goodale et al., 1978; Carruthers and Hafs, 1980;

Peters et al., 1981). Pituitary content of LH increased during the early postpartum period (Saiduddin et al., 1968; Wagner et al., 1969) and responsiveness of the pituitary gland, in terms of LH release after injection of GnRH, is regained between days 10 and 20 postpartum (Kesler et al., 1977; Fernandes et al., 1978b). High concentrations of progesterone and estrogens prepartum have been proposed as a possible cause of refractoriness of the pituitary gland to secrete LH early postpartum. Present results suggest that early postpartum mechanisms of restoration of pituitary function, as assessed by measurements of basal plasma LH concentrations and LH responses to GnRH, were not altered despite appreciable changes in prepartum steroidal profiles between service sire-breed groups (see Chapter VI). Using a similar approach, Eley et al. (1981b) also failed to detect differences in the ability of the pituitary gland to release LH postpartum in two groups of Jersey cows that showed significant hormonal alterations prepartum.

Injection of GnRH in postpartum cows was accompanied subsequently by an increase in plasma concentrations of progesterone due to formation of a corpus luteum or luteinized follicles (Britt et al., 1974; Kesler et al., 1978; Eley et al., 1981b). Although the difference was not statistically significant, timing of the increases in plasma progesterone concentrations, following a GnRH-TRH injection, tended to be earlier and was more synchronized (smaller standard error of the mean) in the Angus service sire group than in the Holstein or Brahman service sire groups. All heifers in the Angus service sire group (7/7) had a rise in progesterone within 9 days following a GnRH-TRH injection in comparison to five of seven (5/7) and only two of seven

(2/7) heifers in the Holstein and Brahman service sire groups, respectively (Table 7.5). This difference in responsiveness of the ovary to a GnRH-TRH injection was further reflected by a higher rate of accumulation of progesterone concentrations, early postpartum, in the Angus service sire group (Figure 7.2). In a previous study, Eley et al. (1981b) provided evidence that differences in ovarian responsiveness may exist between two groups of Jersey cows that exhibited different endocrine profiles prepartum and postpartum. Furthermore, results presented in Chapter V also suggested that ovarian function may be altered early postpartum. In the present experiment, differences in ovarian responsiveness cannot be explained clearly. However, variations in prepartum steroidal profiles, lower postpartum concentrations of PGFM (see Chapter VI), and an easier delivery may all be factors that collectively contribute to the tendency for an earlier and more uniform progesterone response in the Angus service sire group. Such factors may affect ovarian responsiveness to a GnRH-TRH injection by altering ovarian follicular growth and maturity as suggested by Kesler et al. (1978).

Early postpartum, uterine horn diameter may reflect uterine growth that occurred during gestation since uterine horn diameter and uterine weight are related closely during this period (Gier and Marion, 1968). During the first two weeks postpartum, cervical and uterine horns diameters were appreciably smaller in the Angus service sire group than in the Holstein and Brahman service sire groups. The smaller uterine accomodation in the Angus service sire group was related also to a lower calf birth weight in this group (see Chapter VI). Sir John Hammond (1927) indicated that weight of the uterus increased steadily during gestation in cattle. It was reported also that uterine accomodation is characterized by cellular hyperplasia during the early stage of gestation (up to day 60) and by cellular hypertrophy and uterine stretching thereafter and up to term (Hammond, 1927; Hutchinson, 1962). Physical contact of the chorion with the uterine epithelium appear to be the primary stimulus of uterine growth (Amoroso, 1952). This is further substantiated by the limited growth of the nongravid uterine horn in cattle and sheep or of the ligated horn in unilaterally pregnant ewes (Bazer et al., 1979) and cattle (Bartol, 1983). Reynolds (1949) stated that uterine growth may be dependent upon corpus luteum activity during gestation. In Chapter VI, the lower prepartum concentrations of progesterone in the Angus service sire group suggested that the conceptus may regulate synthesis of progesterone by the corpus luteum possibly via a gonadotropin-like protein of conceptus origin (Ailenberg and Shemesh, 1983). One of the possible roles of such conceptus regulation of progesterone synthesis may be to control uterine growth and uterine secretion by the maternal environment. In nonpregnant ewes, the period of rapidly increasing protein secretions can be simulated by giving progesterone for 120 days (Moffatt et al., 1980) and coincides with a period of rapidly increasing placental progesterone synthesis (Ricketts and Flint, 1980) and rising plasma progesterone levels (Basset et al., 1969; Tsang, 1978). Furthermore, the rapid rise in protein secretion rate in the intercaruncular endometrium from day 56 of gestation onwards, in the sheep, is accompanied by a marked rise in nuclear progesterone receptor level (Miller et al., 1983) and corresponds to a period of rapid growth of this tissue (Miller and Stone, 1981). Smaller cervical and uterine horn diameters in the Angus service sire group may reflect a smaller uterine growth in response to a dampened conceptus-induced stimulation of progesterone synthesis by the corpus luteum. As compared to the Angus service sire group, priming of the uterus with more progesterone in the Brahman and Holstein service sire groups may have elicited an increased uterine growth and perhaps uterine secretion in response to physical stimulation by the conceptus (chorion).

Postpartum changes in cervical and uterine horn diameters were closely correlated in the present experiment (r = 0.82) and agree with the observations of Fonseca et al. (1983). Average days to cervical and uterine involution, defined as the day postpartum when diameter of the cervix or of the uterine horns no longer decrease were 30 to 32 days, respectively. This is in agreement with Lewis et al. (1984) and within the range of intervals from parturition to complete uterine involution presented in a review by Kiracofe (1980). Although days to cervical and uterine involution did not differ between service sire-breed groups, the absolute sizes and daily rate of reduction in both cervical and uterine horn diameters were appreciably higher in the Holstein and Brahman service sire groups than in the Angus service sire group (Figures 7.3 and 7.4). A greater degree of uterine distension in response to larger calf birth weight in the Holstein and Brahman service sire groups may be related to a faster rate of uterine involution early postpartum. Wray (1982) indicated that the preponderant stimulus to initiate postpartum uterine involution, in the rat, was the loss of distension from the uterine wall. However, Lewis et al. (1984) reported a faster uterine involution, as assessed by an earlier return of the uterus to its nongravid position, in a group of cows that delivered smaller calves. This suggests that factors, other than the uterine distension that is associated with calf birth weight, may affect the rate of cervical and uterine involution.

Presumably, the larger cervical and uterine horns diameters reflected a heavier mass of uterine tissue in the Holstein and Brahman service sire group than in the Angus service sire group. Therefore, a faster reduction in cervical and uterine horns diameters would be associated with an increased rate of tissue loss either in the Brahman and Holstein service sire groups. Marked structural changes in the uterine wall and more specifically in the myometrium characterize the early postpartum period in cattle. The postpartum reduction in uterine mass involved a rapid decrease in both size and number of myometrial cells in the cow (Riesen et al., 1968) and an important loss of collagen as documented in the rat (Harkness and Harkness, 1956; Harkness and Moralee, 1956; Woessner, 1969; Wray, 1982). The large positive venous-arterial differences in α-amino nitrogen measured across the postpartum sheep uterus (D.H. Barron, unpublished observations) probably reflect this important tissue loss. Wray (1982) proposed that the hormonal environment may govern the rate at which collagen loss and involution proceed. Stimulatory effects of suckling (Riesen et al., 1968; Lauderdale et al., 1968) or of injection with GnRH (Britt et al., 1974; Fernandes et al., 1978b) on uterine involution would favor such a proposal. However, in the present study, neither of these stimuli (e.g., suckling and GnRH injection) can account for the faster rate of uterine involution observed in the Holstein and the Brahman service sire groups. Heifers were not suckled and milking and GnRH injection procedures were uniform across the three service sire-breed groups.

Results presented in Chapter V indicated that suppression of $PGF_{2\alpha}$ production early postpartum did not affect the rate of uterine involution, suggesting that $PGF_{2\alpha}$ is not an obligatory factor in this process. However, higher magnitude of postpartum PGFM concentrations (Lewis et al., 1984) and longer duration of PGFM release postpartum (Lindell et al., 1982) have been related to faster uterine involution. Similarly, in the present experiment, appreciably higher profiles of postpartum PGFM concentrations in Brahman and Holstein service sire groups (see Chapter VI) were associated with a faster rate of uterine involution. Collectively, these results suggest that although $PGF_{2\alpha}$ is not an obligatory factor in the process of uterine involution, higher production of $PGF_{2\alpha}$ by the uterus may be associated with it. Recently, Rodemann et al. (1982a) provided evidence that arachidonic acid and its metabolites (PGE_2 and $PGF_{2\alpha}$) may be involved in the acceleration of muscle protein turnover in various physiological and pathological states (e.g., fever, trauma, injury). Calcium appears to promote protein breakdown by stimulating release of both PGE_2 and $PGF_{2\alpha}$ by the muscles cells which in turn activates the lysosomal apparatus (Rodemann et al., 1982b). Involution of the uterus may involve a similar mechanism and a higher uterine production of prostaglandins, possibly $PGF_{2\alpha}$, may accelerate the rate of protein breakdown and enhance uterine involution. An alternative explanation may be that higher uterine production of $PGF_{2\alpha}$ favors uterine involution by its stimulatory effect on smooth muscle contraction as proposed by Lindell et al. (1982). Gier and Marion (1968) stated that the rapid decrease in uterine size was due to vasoconstriction and muscular contraction.

In conclusion, results of the present experiment provide additional support to the concept that prepartum bovine conceptus and maternal interactions induce periparturient endocrine changes of the maternal unit that may influence postpartum reproductive performances. Such carry-over effects into the postpartum period appear to be localized mainly at the ovarian and uterine level. However, possible carry-over effects on lipid metabolism, lactation and pituitary function need to be examined in more detail.

CHAPTER VIII
GENERAL DISCUSSION

Dynamic endocrine and physiological changes characterize the periparturient period in cattle. At this time, the conceptus unit becomes fully mature and initiates a cascade of endocrine events that lead ultimately to the process of parturition. This is reflected in the maternal unit by changes in various hormones concentrations such as plasma progesterone (Smith et al., 1973; Hoffmann et al., 1973; Eley et al., 1981a; Collier et al., 1982) and estrogens (Robinson et al., 1971; Smith et al., 1973; Comline et al., 1974; Eley et al., 1981a; Collier et al., 1982). In association with the initiation of the process of parturition, peripheral concentrations of PGFM increase, reach a peak by day 2 or 3 postpartum, and returns to basal concentrations by approximately day 15 postpartum (Edqvist et al., 1978; Eley et al., 1981b; Lindell et al., 1982). During the postpartum period, the large uterus undergoes an involutionary phase (Marion et al., 1968; Casida et al., 1968) and ovarian function is resumed usually within 3 weeks postpartum in dairy cattle (Gier and Marion, 1968). The present research provides evidence that periparturient endocrine and physiological changes are interrelated and may reflect prepartum interactions between conceptus and maternal units.

An experimental model, in which Holstein heifers were bred to either Holstein, Brahman, or Angus bulls, was used to delineate conceptus and maternal effects on responses measured during the periparturient period. Changes in maternal endocrine and physiological responses were due primarily to breed of the service sire and provided a sensitive estimate of conceptus genotype effects. Earlier experiments, involving reciprocal crosses between breeds of animals that varied largely in size (Walton and Hammond, 1938; Joubert and Hammond, 1958), stressed the importance of the maternal environment in regulating fetal growth. Eley et al. (1981a; 1981b) demonstrated that genotype of the conceptus may influence the endocrine balance of the maternal unit, but effects of the sire of fetus and of sire of dam were confounded. Results presented in Chapter VI and VII indicated that variation in fetal genotype, that were associated primarily with variation of the breed of sire of fetus influenced endocrine and physiological responses of the maternal unit during the periparturient period. This may represent environmental influences imposed on the maternal unit by the conceptus.

Indeed, influences of the conceptus were detected on concentrations of hormones of both maternal and conceptus origins during late gestation. Concentrations of progesterone measured in peripheral circulation are primarily of maternal origin in pregnant cattle. The corpus luteum appears to be the major source of progesterone (Thorburn et al., 1977) whereas contribution by the placenta was relatively minor (Comline et al., 1974; Wagner et al., 1974; Hoffmann et al., 1976; Ferrell et al., 1983). The prepartum decreases in profiles of progesterone concentrations presented in Chapter VI were in agreement with previous observations (Smith et al., 1973; Hoffmann et al., 1973; Eley et al., 1981a; Collier et al., 1982). Yet, differences in profile of prepartum progesterone concentrations between service sire-breed groups illustrated an effect of the conceptus on a hormone produced primarily by the maternal unit during gestation. Some evidence indicates that the conceptus may influence corpus luteum function during pregnancy in cattle. Anderson et al. (1979) showed that corpus luteum function was not exclusively dependent upon LH secretion by the maternal pituitary. Hypophyseal stalk transection of beef heifers in midpregnancy did not alter luteal function and pregnancy was maintained. Ailenberg and Shemesh (1983) reported the presence of a chorionic gonadotropin-like protein in extracts of bovine cotyledons. The lower profile of progesterone concentrations in the Angus service sire group may reflect a reduced luteal function associated with a lower production of a chorionic gonadotropin-like protein by conceptus of this genotype. On the other hand, Shemesh et al. (1983) also found a factor, unrelated to the one described by Ailenberg and Shemesh (1983) that inhibits basal progesterone secretion in extracts of both fetal and maternal bovine placental tissues. They suggested that this inhibitory factor, may not reduce progesterone synthesis but rather enhances progesterone metabolism or catabolism to other steroids. Therefore, it is possible that the lower progesterone concentrations in the Angus service sire group, than in the Holstein and Brahman service sire groups may be related to an increase metabolism of progesterone in this group. In such an eventuality, the appreciably lower concentrations of estrogens in the Angus service sire group, than in the Holstein and Brahman service sire groups, also suggest that increased metabolism of progesterone in the Angus service sire group would be in favor of steroids other than estrogens.

Uterine growth is stimulated by the presence of the conceptus (Amoroso, 1952) and involves cellular hyperplasia during the early stage of gestation and cellular hypertrophy and uterine stretching from 60 days of gestation onward in cattle (Hammond, 1927; Hutchinson, 1962). The limited growth of the nongravid uterine horn in cattle and sheep or of the ligated horn in unilaterally pregnant ewes (Bazer et al., 1979) and cattle (Bartol, 1983) emphasizes the importance of the physical contact of the chorion with the uterine epithelium to stimulate uterine growth. Reynolds (1949) stated that corpus luteum activity may affect uterine growth, and uterine secretions can be stimulated by progesterone (Moffatt et al., 1980; Ricketts and Flint, 1980). As compared to the Holstein and Brahman service sire groups, calf birth weight was lower in the Angus service sire group and was associated with an appreciably smaller cervical and uterine horn diameter early postpartum (Chapters VI and VII). This may reflect a reduced uterine growth during gestation that was associated with the lower progesterone concentrations measured in the Angus service sire group. Priming of the uterus with more progesterone in the Holstein and Brahman service sire groups than in the Angus service sire group may have elicited an increased uterine growth and perhaps uterine secretion in response to physical stimulation by the conceptus (chorion). Therefore, mechanism of uterine accomodation may be influenced by the conceptus itself via its control over progesterone concentrations in the maternal unit. This would assure a proper uterine environment that is compatible with the rate of growth of the conceptus dictated by the genotype of the conceptus.

Studies of concentration gradients across the bovine uterus indicated that estrogens are produced by tissue within the gravid uterus (Comline et al., 1974; Peterson et al., 1975; Ferrell and Ford, 1980; Ferrell et al., 1983). Ainsworth and Ryan (1966) isolated an active aromatase system in placental tissue, and Hoffmann et al. (1976; 1979) indicated that the bovine placentome is the major source of estrogens near term. Estrone and estradiol-17_{β} are the major estrogens produced, and a significant portion is conjugated at the site of synthesis, in the fetal cotyledon. Increasing prepartum plasma concentrations of estrogens (estrone, estradiol and estrone sulfate) presented in Chapter VI were in agreement with previous observations (Robinson et al., 1971; Smith et al., 1973; Comline et al., 1974; Eley et al., 1981a; Collier et al., 1982). Predominance of plasma concentrations of estrone sulfate over those of unconjugated estrone and estradiol in maternal circulation also agreed with reports by Tsang et al. (1975), Robertson and King (1979), Hoffmann et al. (1979), Eley et al. (1979) and Eley et al. (1981a). Alterations in plasma estrogens concentrations between service sire-breed groups in the present study were due primarily to the sire of fetus and reflect a degree of control by the conceptus on a product of its own.

One of the most important functions of the placenta is to achieve transfer of respiratory gases and nutrients between maternal and fetal circulation. Maturational changes of the the placental function to accomodate the faster growing fetus have been reported by Battaglia et al. (1965), Kulhanek et al. (1974) and Longo and Ching (1977). Thatcher et al. (1980) suggested that estrogen concentrations may represent the capacity of the conceptus to regulate its intrauterine environment to achieve growth. Collier et al. (1982) showed that concentrations of estrone sulfate and calf birth weight were reduced when cows were heat stressed during the last trimester of gestation. Furthermore, calf birth weight (Collier et al., 1980) and bovine placental weight (Head et al., 1981) were lower during the hot summer months. In the present study, the lower prepartum concentrations of estrogens in the Angus service sire group were associated with lower calf birth weights. In agreement with Terqui et al. (1975), concentrations of estrogens were correlated positively with calf birth weight. Collectively, these results suggested that measurement of prepartum maternal concentrations of estrogens, and primarily of the predominant estrone sulfate, may be a functional index of the fetal part of the placentome. Jorgenson and Frandsen (1973) established a relationship between low estrogens and compromised fetal wellbeing in human. In heat stressed cattle (Collier et al., 1982), the low estrogen concentrations may have been associated with reduced metabolism by the conceptus to overcome overheating problems within the uterus. In the present study, the differential prepartum profiles of estrogen concentrations between service sire-breed groups may represent the capacity of the conceptus to regulate its intrauterine environment to achieve growth at the rate imposed by its genetic code. That the differences in maternal estrogen concentrations were due primarily to the sire of fetus also illustrated a degree of control by the conceptus on the maternal unit.

The influence of the sire of fetus on calf birth weight was recognized by Joubert and Hammond (1958) and Boyd and Hafs (1965). Osinga (1978) indicated that the sire or breed of the sire of fetus influenced the urinary estrogen/creatinine ratio during late pregnancy in cows. The present study showed that within service sire-breed groups, mean concentrations of estrone sulfate and estradiol were the only responses that were affected significantly by the service sire. This would be anticipated since estrone sulfate is the major product of conceptus origin measured in maternal circulation. This further exemplifies the inherent variability and importance of the service sire that is reflected in the maternal unit via a product of the conceptus. This provides additional support to the concept that variation in estrogens concentrations or other products of conceptus origin (e.g., bovine placental lactogen, chorionic gonadotropin-like protein) may be the mechanism by which sire of fetus exerts its influences on calving ease, frequency of dystocia (Quesnel et al., 1979) or on subsequent milk and fat yield of the dam after calving (Adkinson et al., 1977; Thatcher et al., 1980).

Concentrations of PGFM, the primary metabolite of $PGF_{2\alpha}$, increased at the time of parturition and remained elevated for approximately 15 days postpartum in dairy cattle. The tissue producing $PGF_{2\alpha}$ was unknown since only peripheral concentrations of PGFM (Edqvist et al., 1978; Eley et al., 1981b; Lindell et al., 1982) or of uterine venous $PGF_{2\alpha}$ (Fairclough et al., 1975) were measured. Different experimental approaches (Chapter III and IV) clearly established that the bovine uterus is the major source of F series prostaglandins during the early postpartum period. Hysterectomy, performed on the day of parturition, resulted in a precipitous decline in PGFM concentrations and completely eliminated the postpartum elevation in peripheral PGFM concentrations. Large venous-arterial concentration gradients of F series prostaglandins were measured across the postpartum bovine uterus and capability of postpartum bovine intrauterine tissues to produce F series prostaglandins was demonstrated.

Changes in uterine blood flow and in degree of synthesis and metabolism of $PGF_{2\alpha}$ within the uterus are two factors that contribute to variations in concentrations of PGFM measured in the peripheral circulation. Results presented in Chapter IV indicated that PGFM concentrations in the uterine artery were correlated positively with uterine venous, and venous-arterial differences in concentrations of $PGF_{2\alpha}$, PGFM and of total F series prostaglandins ($PGF_{2\alpha}$ + PGFM). Furthermore, there was a positive correlation between PGFM concentrations in the uterine artery and estimates of daily production of $PGF_{2\alpha}$ and total F series prostaglandins ($PGF_{2\alpha}$ + PGFM). Therefore, these results provide strong evidence that concentrations of PGFM in the uterine artery or an equivalent peripheral sample is a good indicator of synthesis and metabolism of $PGF_{2\alpha}$ within the uterus and a reliable index of daily uterine production of these prostaglandins in postpartum cows. A similar relationship was found in cyclic cows after uterine production of F series prostaglandins had been stimulated with an injection of estradiol (Knickerbocker et al., 1984). The early postpartum (days 1 to 5) decrease in estimates of daily production of F series prostaglandins was associated primarily with a large decrease in uterine blood flow. At the level of the uterus, large positive venous-arterial differences in concentrations of $PGF_{2\alpha}$ and PGFM were maintained during this period.

Maintenance of large and sustained positive venous-arterial differences in $PGF_{2\alpha}$ and PGFM reflects the high degree of synthesis and metabolism of $PGF_{2\alpha}$ that prevails during the early postpartum period. This contrasts largely with the low concentrations gradients of $PGF_{2\alpha}$ and PGFM measured across the bovine uterus during late gestation (Gimenez et al., 1983). Therefore, activation of enzymes responsible for synthesis (phospholipase A_2 and cyclo-oxygenase; Ramwell et al., 1977) and metabolism (15-hydroxy prostaglandin dehydrogenase and prostaglandin Δ-13 reductase; Anggard et al., 1971) of $PGF_{2\alpha}$ coincided with delivery of the feto-placental unit. Furthermore, examination of the dynamics of in vivo synthesis and metabolism of $PGF_{2\alpha}$ within the early postpartum bovine uterus (Chapter IV) indicated that metabolism of $PGF_{2\alpha}$ did not occur at the expense of a pool of $PGF_{2\alpha}$ but was rather dependent upon continual synthesis of $PGF_{2\alpha}$ within the uterus.

Higher degrees of synthesis and metabolism of F series prostaglandins in caruncular and intercaruncular endometrium than in the myometrium was in agreement with previous observations in sheep (Michell and Flint, 1978b) and goat (Mitchell et al., 1978) around the time of parturition. Total quantity of F series prostaglandins released into the culture medium (Chapter III) tended to be higher for the caruncular than the intercaruncular endometrium, but the major difference between the two most active intrauterine tissues resided in the greater ability of the caruncular endometrium to metabolize $PGF_{2\alpha}$ to PGFM.

Keirse et al. (1976; 1977) suggested that the periparturient increase in activity of the 15-hydroxy prostaglandin dehydrogenase in sheep maternal cotyledonary tissue (caruncle) may reflect a high degree of prostaglandin synthetic activity within this tissue. There are evidences that synthesis of arachidonic acid, the precursor of $PGF_{2\alpha}$, PGFM and PGE_2 synthesis (Pace-Aciak, 1977), occurs within the placentome cotyledon (maternal and fetal) of the sheep (Christie and Noble, 1982). Toward the end of gestation, there was an increase in the capability to synthesize arachidonic acid within the sheep placentome (Shand and Noble, 1981); at this time, proportion of arachidonic acid within this tissue was higher than in any of the maternal plasma lipid fractions (Noble et al., 1982). Collectively, these results suggest that in the ruminant, the prepartum placentome (fetal and maternal) and residual placentome tissue postpartum (maternal caruncle) are likely to be a major source of arachidonic acid substrate that is available for the high degree of prostaglandin synthesis and metabolism within this tissue. Shemesh et al. (1981) reported the presence in term bovine fetal cotyledonary tissue of a cytosolic protein that is a potent inhibitor of prostaglandin synthetase. Removal of fetal cotyledonary inhibition at parturition (delivery of calf and fetal placenta) would likely increase production of $PGF_{2\alpha}$ and its subsequent metabolism by the richly vascularized maternal caruncle. Capability of bovine arteries and veins to synthesize $PGF_{2\alpha}$ was demonstrated previously (Terragno et al., 1978).

Persistence of high concentrations of PGFM for an extended period of time postpartum appears to be unique to the ruminant (cow and sheep). In horses (Allen and Pashen, 1981) and pigs (Nara and First, 1981) which have a different type of placentation (microcotyledonary and diffuse placentation, respectively; Steven, 1975), concentrations of PGFM peaked at parturition and decreased rapidly to basal level within 24 h. In sheep, which also have a cotyledonary-caruncular type of placental attachment, the situation is intermediary when compared with that of the cow; the rate of decline of postpartum PGFM concentrations was greater than that reported for cows and by day 11 postpartum, PGFM concentrations were basal (Lewis and Bolt, 1983). Present research characterized the bovine uterus as the major source of F series prostaglandins, and the caruncles as a very active site of synthesis and metabolism of $PGF_{2\alpha}$. This introduces the possibility that postpartum uterine production and metabolism of $PGF_{2\alpha}$ in cattle, may be affected by the degree of fetal and maternal interactions that occurred during gestation.

Caruncular growth is stimulated by the presence of the conceptus (Amoroso, 1952; Alexander, 1964) and, as a result, nutrition of the fetus is assured by "assisted transfusion between maternal and fetal blood streams in the placentome" (Hammond, 1961). Compensatory growth of the fetal and/or maternal part of the placentome following physical alteration of its development (Alexander, 1964; Emmanouilides et al., 1968; Hobel et al., 1970; Metcalfe, 1970; Cefalo et al., 1977; Clapp et al., 1981; Caton et al., 1983) reflected a real interaction between fetal and maternal units. These morphological changes that were induced in an attempt to provide optimal conditions for fetal development were expressed microscopically by changes in surface area of the placenta (Smith et al., 1983) or in maternal and fetal placental vascularization (Van der Heijden, 1981). Different profiles of postpartum PGFM between service sire-breed groups that were presented in Chapter VI, may represent an environmental effect of the conceptus on the maternal unit (caruncle). Genetically different conceptus groups caused a differential stimulation of the maternal unit which in turn responded by providing a sufficient intrauterine environment (caruncles) that permitted ultimate growth of the fetus to its programmed genetic size. However, this conceptus-induced environmental effect, as measured by plasma PGFM concentrations, is not expressed until the postpartum period. In a previous experiment, concentrations of PGFM were higher postpartum in cows heat stressed during the prepartum period than in those cows managed under a shade area during the same period (Lewis et al., 1984). This may reflect an increased caruncular development and/or vascularization that occurred in compensation for the decrease uterine blood flow in heat stressed cows during gestation. Therefore, these results suggest that postpartum peripheral concentrations of PGFM may be a functional index of the maternal part of the placentome. The author feels that this response is expressed primarily by the magnitude of PGFM concentrations early postpartum. Magnitude of the response, at this time, reflects the caruncular mass before it becomes necrotic at approximately day 5 postpartum (Marion and Gier, 1968).

Restoration of postpartum reproductive efficiency is characterized by reestablishment of a functional hypothalamo-pituitary-ovarian axis, ovarian follicular growth (Stevenson and Britt, 1980) and uterine involution (Gier and Marion, 1980). Results presented in Chapter V and VII showed that some of these processes may be influenced by the conceptus-induced changes of the maternal unit that occurred during the periparturient period in cattle. However, such effects appear to be localized mainly at the ovarian and uterine level.

Inspite of variation in periparturient endocrine profiles of the maternal unit, this study as well as that by Eley et al. (1981b) failed to detect differences in the ability of the pituitary gland to release LH postpartum after a GnRH-TRH challenge.

Eley et al. (1981b) introduced the possibility that postpartum ovarian responsiveness may be altered by periparturient endocrine changes in cattle. Differences in postpartum ovarian responsiveness also were detected between service sire-breed groups in the present study (Chapter VII). Although the mechanism is still unknown, variation in prepartum steroidal profiles, ease of parturition and lower postpartum concentrations of PGFM may all be factors that collectively contribute to the tendency for an earlier and more uniform progesterone response to a GnRH-TRH injection in the Angus service sire group.

A possible role of $PGF_{2\alpha}$ on postpartum ovarian function was investigated in Chapter V. Results indicated that early postpartum suppression of endogenous prostaglandin synthesis had deleterious effects on ovarian function. While postpartum ovarian activity of both ovaries was reduced by suppression of prostaglandin synthesis, replacement therapy with an infusion of $PGF_{2\alpha}$, early postpartum, had a beneficial effect specifically on ovaries ipsilateral to the previously gravid uterine horn. This beneficial effect of $PGF_{2\alpha}$ was associated also with greater progesterone production during the first 60 days postpartum. Although the general decrease in ovarian activity after suppression of prostaglandin may be ascribed to a lower secretion of pituitary LH or perhaps to impaired follicular development, the specific response of ovaries ipsilateral to the previously gravid uterine horn to an infusion of $PGF_{2\alpha}$ probably reflects a local effect of uterine $PGF_{2\alpha}$ on the ovary and/or the adjacent uterine horn. As compared to a group of cows that only received physiological saline, postpartum replacement therapy with an infusion of $PGF_{2\alpha}$ was associated with a faster decrease in ovarian volume of ovaries ipsilateral to the previously gravid uterine horn. This suggests that postpartum uterine $PGF_{2\alpha}$ may enhance follicular development by causing a faster demise of the CL of pregnancy. Casida and Vengke (1936) and Foote and Peterson (1968) indicated that the CL of pregnancy may slow the process of folliculogenesis early postpartum. Another possibility may be that uterine $PGF_{2\alpha}$ contributes to the reestablishment of an ovarian blood flow that is compatible with proper ovarian function. Lamond and Drost (1974) proposed that the large postpartum blood supply to the ovary, via the UBOA, may exceed its need and become detrimental to ovarian function. Uterine $PGF_{2\alpha}$ may regulate postpartum ovarian function by causing vasoconstriction of the UBOA thereby contributing to reestablishment of an adequate blood flow to the ovary ipsilateral to the previously gravid uterine horn. Lewis et al. (1984) also detected an enhanced ovarian activity on ovaries ipsilateral to the previously gravid uterine horn that was associated with higher PGFM concentrations postpartum in cows heat stressed during the last trimester of gestation.

There was a positive relationship between the reduction in size of the uterine horn postpartum and concentrations of PGFM in peripheral circulation (Eley et al., 1981b) suggesting a possible role of $PGF_{2\alpha}$ in the process of uterine involution. Suppression of endogenous prostaglandins synthesis early postpartum, in the present study, did not affect the postpartum changes in cervical and uterine horns diameters and in location of the uterus. This indicates that $PGF_{2\alpha}$ is not an obligatory factor in the process of uterine involution. On the other hand, it appears that a postpartum augmentation in $PGF_{2\alpha}$ secretion may enhance the processes of uterine involution. Results presented in Chapters VI and VII indicated that the appreciably higher profiles of postpartum PGFM concentrations in the Brahman and Holstein service sire groups were associated with a faster rate of uterine involution. This is in agreement with previous observations showing that a higher magnitude of postpartum PGFM concentrations (Lewis et al., 1984) and longer postpartum duration of elevated PGFM concentrations (Lindell et al., 1982) were related to a faster uterine involution. It is possible that $PGF_{2\alpha}$ enhances uterine involution by its stimulatory effects on smooth muscle contraction as proposed by Lindell et al. (1982). Alternatively, higher production of uterine prostaglandins, possibly $PGF_{2\alpha}$, may accelerate rate of tissue loss by increasing the rate of protein turnover (Rodemann et al., 1982a; 1982b). This would enhance uterine involution.

Postpartum uterine production of $PGF_{2\alpha}$, in dairy cattle, appears to influence processes involved in resumption of ovarian activity and uterine involution. Early postpartum suppression of $PGF_{2\alpha}$ decreased postpartum ovarian function, but this condition was restored after replacement therapy with $PGF_{2\alpha}$ early postpartum. Although $PGF_{2\alpha}$ is not an obligatory factor in the process of uterine involution, higher concentrations of $PGF_{2\alpha}$ hastened this process. With this knowledge, field trial experiments designed to test the effect of postpartum supplementation with $PGF_{2\alpha}$ to improve postpartum efficiency are warranted. The author feels that such supplementation with $PGF_{2\alpha}$ should be started early postpartum (days 3 to 5), time during which endogenous production of $PGF_{2\alpha}$ is decreasing and major changes in ovarian follicular development and uterine condition are occurring.

The preceeding discussion has indicated several possible mechanisms by which the conceptus may influence the environment in which it grows. The present research has provided additional evidence of interactions between conceptus and maternal units that were associated with specific endocrine and physiological responses. Through these interrelationships, reproductive function and production traits of the dam are potentially influenced by the conceptus.

APPENDIX A

POLYNOMIAL REGRESSION COEFFICIENTS FOR RESPONSES MEASURED IN CHAPTER III

TABLE A.1. Least squares polynomial regression coefficients for PGFM concentrations during experimental phases (pre- and post-replacement or ligation of the uterus and from days 1 to 15 postpartum) in sham-operated, hysterectomized and control groups.

Group[a]	N[b]	B_0	B_1	B_2	B_3	B_4	B_5
Phase I Pre-replacement or Pre-ligation							
Pooled[c]	53	2935.35	264.948X[d]	$-47.1374X^2$	$2.121619X^3$	$-0.0374581X^4$	$0.0002296900X^5$
Phase II Post-replacement or Post-ligation							
S	20	-1815.30	142.007X	$-1.6770X^2$	$0.008241X^3$	$-0.0000182X^4$	$0.0000000149X^5$
H	41	8803.50	-253.534X	$2.8788X^2$	$-0.015222X^3$	$0.0000377X^4$	$-0.0000000352X^5$
C	-	-	-	-	-	-	-
Phase III D1 to D15 Postpartum							
S	75	-612.12	1903.191X	$-700.5658X^2$	$100.507225X^3$	$-6.3399996X^4$	$0.1469775600X^5$
H	134	89.72	0.192X	$-4.1299X^2$	$0.793982X^3$	$-0.0555237X^4$	$0.0013427500X^5$
C	60	327.72	1132.673X	$-316.0315X^2$	$36.725060X^3$	$-2.0724609X^4$	$0.0459650900X^5$

[a] S = sham-operated; H = hysterectomized; C = control.

[b] Total number of observations.

[c] Pooled = sham-operated + hysterectomized; homogeneity of regression, $P > 0.1$.

[d] X = min, phase I and phase II; X = days, phase III.

APPENDIX B

POLYNOMIAL REGRESSION COEFFICIENTS FOR RESPONSES MEASURED IN CHAPTER V

TABLE B.1. Least squares polynomial equations for postpartum dependent variables in three treatment groups by Brown Swiss cows.

Dependent variable	Treatment group	B_0	B_1	B_2	B_3	B_4	B_5
PGFM[a]	FM + PGF$_{2\alpha}$	-164.14	662.53173206X	-81.66880762X^2	2.17847174X^3		
	FM + Saline	-31.79	310.18004750X	-26.83490790X^2	-0.00084803X^3		
	Control	431.43	704.91637624X	-149.64590473X^2	7.62341167X^3		
Accumulated progesterone[a]	FM + PGF$_{2\alpha}$	25.92	-23.07620920X	13.54796381X^2			
	FM + Saline	11.36	-4.85999230X	8.19814017X^2			
	Control	-4.11	16.13941743X	8.93727507X^2			
LH[a]	FM + PGF$_{2\alpha}$	0.5648	0.01005520X	0.03134197X^2	-0.00428566X^3		
	FM + Saline	0.5684	0.08494208X	0.01644006X^2	-0.00502971X^3		
	Control	0.6798	0.56398395X	-0.22711993X^2	0.02364863X^3		
Accumulated estradiol[a]	FM + PGF$_{2\alpha}$	20.78	76.40667434X	-5.14131064X^2			
	FM + Saline	10.87	64.20597833X	-3.79983979X^2			
	Control	-16.74	141.97185522X	-12.24811557X^2			
Cervix diameter[b]	pooled	63.9100	-1.61619948X	-0.11104103X^2	0.00934499X^3	-0.00016087X^4	
Uterine location[b]	pooled	-6.4657	2.34935154X	-0.11053444X^2	0.00168361X^3		
Previously gravid uterine horn[b]	pooled	165.7000	-35.55246596X	4.01153190X^2	-0.22693790X^3	0.00624266X^4	-6.6179391E-05
Previously nongravid uterine horn[b]	pooled	76.08	-11.46163149X	1.18759151X^2	-0.06478558X^3	0.00177310X^4	-1.8934027E-05
Ovarian volume ipsilateral[b]	FM + PGF$_{2\alpha}$	0.3753	5.20124590X	-0.97545752X^2	-0.07466180X^3	-0.00244858X^4	2.8745202E 05
	FM + Saline	-1.5731	9.21649342X	-1.74666173X^2	0.12544444X^3	-0.00387604X^4	4.3511711E-05
	Control	2.1226	5.08778985X	-0.94965164X^2	0.06749989X^3	-0.00209249X^4	2.3862998E-05
Accumulated units of ovarian activity (ipsilateral)[b]	FM + PGF$_{2\alpha}$	0.5002	-0.0481110X	0.01143937X^2			
	FM + Saline	-0.1832	-0.00395996X	0.00700691X^2			
	Control	-1.7160	0.35427631X	-0.00039404X^2			
Accumulated units of ovarian activity (contralateral)[b]	FM + PGF$_{2\alpha}$	-1.4583	0.38156023X	0.00116769X^2			
	FM + Saline	-2.5689	0.56199932X	-0.00295346X^2			
	Control	-2.8420	0.75759183X	-0.00515965X^2			
Accumulated units of CL occurrence (ipsilateral)[b]	FM + PGF$_{2\alpha}$	47.5851	-18.45459051X	1.08563921X^2			
	FM + Saline	-11.8689	-2.33086944X	0.34722968X^2			
	Control	49.2600	-9.23551928X	0.65712113X^2			
Accumulated units of CL occurrence (contralateral)[b]	FM + PGF$_{2\alpha}$	-70.6417	10.02865978X	0.39569587X^2			
	FM + Saline	-76.1536	5.42948330X	0.54466747X^2			
	Control	-184.02	31.14935163X	-0.07948247X^2			

[a] X = coded day postpartum; 1 < X < 6; actual day = coded day x 10.
[b] X = coded day postpartum; 1 < X < 30; actual day = coded day x 2.

APPENDIX C

COMPLEMENTARY ANALYSES AND POLYNOMIAL REGRESSION COEFFICIENTS FOR RESPONSES MEASURED IN CHAPTERS VI AND VII

TABLE C.1. Characteristics of gestation, parturition and postpartum period in Holstein heifers bred to either Holstein, Brahman or Angus bulls.

Service sire group	Characteristic					
	gestation length (days)	conditions of parturition	calf status at parturition	calf sex	calf birth weight (kg)	postpartum uterine status
Holstein						
H-782	284	normal	normal	M	40.0	normal[a]
H-794	277	normal	normal	M	40.9	normal
H-800	282	normal	normal	F	28.6	normal
H-788	277	normal	normal	F	34.1	normal
H-767	280	normal	stillborn	F	37.3	infection
H-781	277	assisted	stillborn	F	37.7	infection
H-789	274	normal	stillborn	F	34.1	infection
Brahman						
H-772	279	normal	normal	M	35.4	normal
H-783	288	assisted	normal	M	43.2	infection
H-778	269	normal	normal	F	35.9	normal[a]
H-793	295	assisted	normal	M	50.0	infection
H-784	291	normal	normal	F	35.9	normal
H-798	285	cesarian section	normal	M	68.2	adhesion[a]
H-780	288	assisted	stillborn	F	35.4	normal
Angus						
H-773	284	normal	normal	F	36.4	normal
H-791	280	normal	normal	M	26.8	normal
H-770	280	normal	normal	M	32.7	infection
H-775	277	normal	normal	M	30.9	normal
H-801	275	normal	normal	F	27.7	normal
H-777	270	normal	stillborn	M	26.4	infection
H-779	287	assisted	stillborn	F	33.6	cystic

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[a] Retained placenta.

TABLE C.2. Tests of homogeneity of regression for periparturient hormonal responses of Holstein heifers that deliver normally (n = 11) and Holstein heifers that experienced clinical problems (dystocia, stillbirth or postpartum uterine infections; n = 10).

Response[a]	Order of regression	Error term		Treatment	
		df[b]	MS[b]	df[c]	MS_D[c]
Prepartum					
estrone	3	325	192392	3	145087
estradiol	3	297	8085	3	12124
estrone sulfate	3	346	17623864	3	94103959**
estrone sulfate[d]	3	218	6126272	3	5514292
Postpartum					
PGFM	3	382	752802004	3	3759834

[a] Mean concentrations were not different for any responses ($P > 0.1$).

[b] Degrees of freedom (df) and mean square (MS) associated with the error term when individual curves were generated for each treatment.

[c] Degrees of freedom (df) and mean square (MS_D) associated with the difference in residuals sums of squares between fitting one pooled curve versus an individual curve for each treatment.

[d] Excluding heifers that were in the Brahman service sire group.

TABLE C.3. Least squares analyses of prepartum and postpartum percent hematocrit and plasma protein concentrations in Holstein heifers bred to either Holstein, Brahman or Angus bulls.

Source of variation	Prepartum				Postpartum				Error Term
	Hematocrit		Plasma protein		Hematocrit		Plamsa protein		
	df	MS	df	MS	df	MS	Df	MS	
Breed	2	44.85	2	1.33	2	53.02	2	9.69	Service sire (breed)
Service sire (breed)	9	48.42	9	1.36	9	70.33	9	7.17	Heifer (service sire, breed)
Heifer (service sire, breed)	9	68.86**	9	2.44**	9	118.88**	9	8.73**	Residual
Days	3	84.46**	3	0.98**	4	529.00**	4	6.91**	Residual
Residual	352	1.71	354	0.09	705	2.88	709	0.25	

**P < 0.01.

TABLE C.4. Tests of homogeneity of regression and orthogonal contrast of prepartum and postpartum percent hematocrit and plasma protein concentrations in Holstein heifers bred to either Holstein, Brahman or Angus bulls.

Response	Order of regression	Error term		Treatment		Orthogonal contrast[c]	
		df[a]	MS[a]	df[b]	MS_D[b]	A	B
Prepartum							
Hematocrit	3	346	1.56	6	10.73**	*	**
Plasma Protein	3	348	0.08	6	0.54**	NS	*
Postpartum							
Hematocrit	4	697	2.71	8	17.85**	**	**
Plasma Protein	4	701	0.22	8	3.15**	**	**

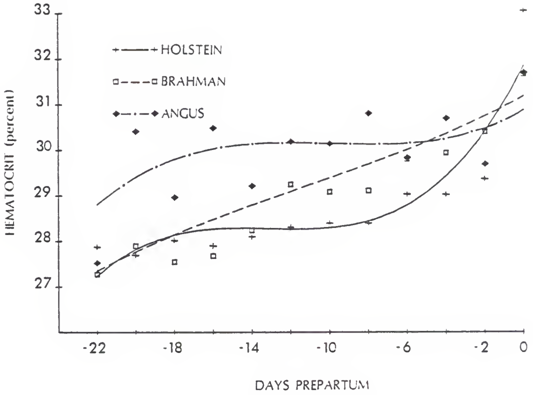
[a] Degrees of freedom (df) and mean square (MS) associated with the error term when individual curves were generated for each treatment.

[b] Degrees of freedom (df) and mean square (MS_D) associated with the difference in residuals sums of squares between fitting one pooled curve versus an individual curve for each treatment.

[c] Orthogonal contrast: A = Holstein, Brahman versus Angus
B = Holstein versus Brahman.

** $P < 0.01$; * $P < 0.05$; NS = not significant.

Figure C.1 Least squares regressions and least square means of prepartum (upper panel) and postpartum (lower panel) percent hematocrit in Holstein heifers bred to either Holstein, Brahman or Angus bulls.


33
32
31
30
29
28
27
HEMATOCRIT (percent)
HOLSTEIN
BRAHMAN
ANGUS
-22
-18
-14
-10
-6
-2
0
DAYS PREPARTUM

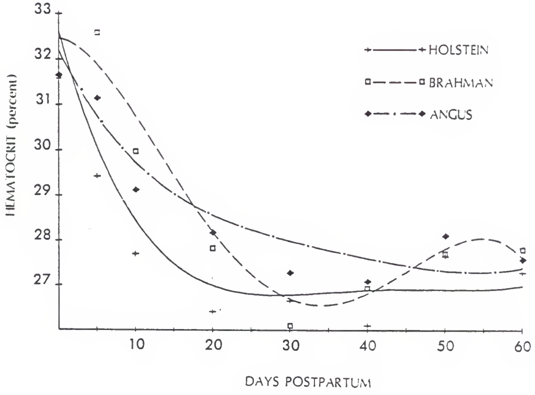
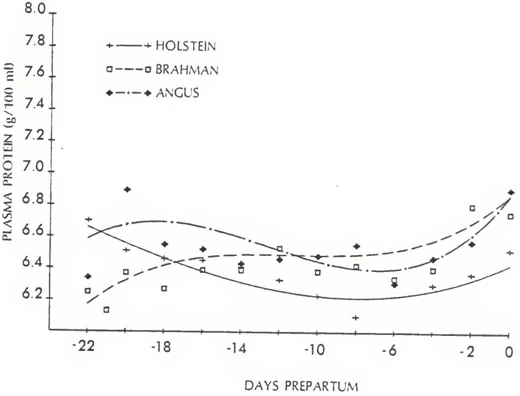

33
32
31
30
29
28
27
HEMATOCRIT (percent)
HOLSTEIN
BRAHMAN
ANGUS
10
20
30
40
50
60
DAYS POSTPARTUM

Figure C.2 Least squares regressions and least square means of prepartum (upper panel) and postpartum (lower panel) plasma protein (g/100 ml) in Holstein heifers bred to either Holstein, Brahman or Angus bulls.


8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
PLASMA PROTEIN (g/100 ml)
HOLSTEIN
BRAHMAN
ANGUS
-22
-18
-14
-10
-6
-2
0
DAYS PREPARTUM

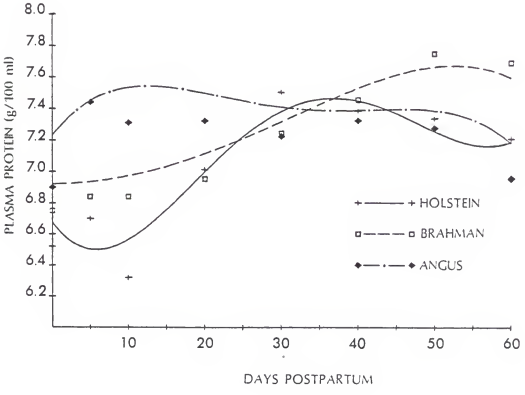

8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
PLASMA PROTEIN (g/100 ml)
HOLSTEIN
BRAHMAN
ANGUS
10
20
30
40
50
60
DAYS POSTPARTUM

TABLE C.5. Least squares polynomial equations for prepartum dependent variables in Holstein heifers bred to either Holstein, Brahman or Angus bulls.

Dependent variables	Service sire-breed group	B_0	B_1	B_2	B_3
Hematocrit	Holstein	-2283.58	614.81470850X[a]	$-54.49246257X^2$	$1.60966355X^3$
	Brahman	-315.03	87.10575697X	$-7.46697775X^2$	$0.21701222X^3$
	Angus	-1308.22	346.37611988X	$-29.87022025X^2$	$0.85830104X^3$
Plasma Proteins	Holstein	-32.75	13.37252070X	$-1.42694702X^2$	$0.04860453X^3$
	Brahman	-412.97	109.78920387X	$-9.57686967X^2$	$0.27840628X^3$
	Angus	-570.52	153.56886201X	$-13.58767660X^2$	$0.39972288X^3$
Progesterone	Holstein	1839559.00	-495001.75351663X	$44545.23331609X^2$	$-1336.55373762X^3$
	Brahman	2007762.00	-543391.13035293X	$49116.60375285X^2$	$-1478.45320136X^3$
	Angus	1426551.00	-380190.90384413X	$33933.45758356X^2$	$-1010.24972254X^3$
Estrone	Holstein	1045010.43	-271481.19345121X	$23407.25558590X^2$	$-669.36289587X^3$
	Brahman	1263352.46	-324309.08731442X	$27618.64292185X^2$	$-779.74364417X^3$
	Angus	791426.76	-207075.65135266X	$17993.64759142X^2$	$-518.86713534X^3$
Estradiol	Holstein	107628.42	-27906.85292338X	$2401.56522230X^2$	$-68.52735119X^3$
	Brahman	6109.09	-702.76588573X	$-26.07289166X^2$	$3.61450607X^3$
	Angus	109489.07	-28479.59767006X	$2461.14672831X^2$	$-70.59958929X^3$
Estrone sulfate	Holstein	5677842.84	-1511620.97145607X	$133961.09725042X^2$	$-3943.29801409X^3$
	Brahman	2892642.08	-777216.35983289X	$69176.71408911X^2$	$-2030.81535422X^3$
	Angus	1883650.97	-514520.27128221X	$46789.95073685X^2$	$-1410.47737813X^3$

[a] X = coded day prepartum, $10.2 < X < 12.7$; actual day prepartum = -10 (12.6 - coded day).

TABLE C.6. Least squares polynomial equations for postpartum dependent variables in Holstein heifers bred to either Holstein, Brahman or Angus bulls.

Dependent variable	Service sire-breed group	B_0	B_1	B_2	B_3	B_4
Hematocrit[a]	Holstein	32.62	$-6.17730203X^a$	$2.35403457X^2$	$-0.38252840X^3$	$0.02261554X^4$
	Brahman	32.47	$-0.33505247X$	$-1.97853351X^2$	$0.64824051X^3$	$-0.05528564X^4$
	Angus	32.19	$-3.42576451X$	$1.14486740X^2$	$-0.19623459X^3$	$0.01306044X^4$
Plasma Protein[a]	Holstein	6.67	$-0.66266721X$	$0.71848939X^2$	$-0.18054349X^3$	$0.01359050X^4$
	Brahman	6.92	$0.01215916X$	$0.03762892X^2$	$0.00491228X^3$	$-0.00140584X^4$
	Angus	7.23	$0.61304120X$	$-0.39304237X^2$	$0.09063584X^3$	$-0.00707032X^4$
PGFM[b]	Holstein	-3825097.66	$854245.53479577X$	$-63407.97098793X^2$	$1564.66423665X^3$	-
	Brahman	-5425913.58	$1213979.58616266X$	$-90282.81392143X^2$	$2232.22016018X^3$	-
	Angus	-1965206.20	$436707.35149338X$	$-32258.01443203X^2$	$792.26057952X^3$	-
LH(GnRH-TRH challenger)[c]	pooled	-9.01	$2.52729320X$	$-0.16287647X^2$	$0.00404570X^3$	$-3.5030880E-05X^4$
Progesterone accumulated[a]	Holstein	2742.00	$1720.55503531X$	$1319.67918346X^2$	-	-
	Brahman	2420.00	$1423.30319879X$	$1336.16027133X^2$	-	-
	Angus	-748.00	$6038.45404568X$	$701.70929899X^2$	-	-
Cervical diameter[d]	Holstein	133.62	$-17.85996486X$	$1.34404296X^2$	$-0.04668569X^3$	$0.00060056X^4$
	Brahman	129.02	$-14.70708691X$	$0.92758233X^2$	$-0.02780177X^3$	$0.00326100X^4$
	Angus	115.39	$-17.05619725X$	$1.38361041X^2$	$-0.04865484X^3$	$0.00616750X^4$
Mean uterine horn diameter[d]	Holstein	127.86	$-18.71751912X$	$1.34917941X^2$	$-0.04319751X^3$	$0.00051531X^4$
	Brahman	133.35	$-19.14813611X$	$1.33564263X^2$	$-0.04124711X^3$	$0.00046817X^4$
	Angus	99.82	$-14.51783265X$	$1.12915135X^2$	$-0.03847333X^3$	$0.00047575X^4$

[a] X = coded day postpartum, $12.5 < X < 18.6$; actual day postpartum = 10 (coded day - 12.6); if actual day postpartum > 15 and actual day is odd, then actual day postpartum = 10 (coded day - 12.6) + 1.

[b] X = coded day prepartum and postpartum, $12.3 < X < 14.1$; coded day of parturition = 12.6, uncoded day of parturition = 0.

[c] X = coded minute after GnRH-TRH injection, $6 < X < 43$; actual minute = 10 (coded minute - 6).

[d] X = coded day postpartum, $12.8 < X < 18.6$; actual day postpartum = 10 (coded day - 12.6); if actual day is odd, then actual day postpartum = 10 (coded day - 12.6) + 1.

TABLE C.7. Means (± S.E.M.) of milk yield, fat yield and days open of Holstein heifers bred to either Holstein, Brahman or Angus bulls.

	Service sire-breed group		
	Holstein	Brahman	Angus
Milk yield (kg) (305,2x,ME)	6229±290	5704±228	6322±569
Fat yield (kg)	214±7.3	201±7	218±25
Days open	137±38	96±16	102±16

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BIOGRAPHICAL SKETCH

Louis Andre Guilbault, son of Marie-Berthe Massicotte and the late Paul Guilbault, was born in the city of Quebec, Canada, on April 28, 1952. He attended public schools in Grondines and Quebec City throughout his primary and secondary education. In 1970, he entered college and received a "Diplome d'Etudes Collegiales en Science" from the College Notre-Dame-de-Foy, Cap-Rouge, Quebec, in June 1973. He attended l'Universite Laval in Quebec City and received a "Bacchalaureat en Science" degree in biology in June 1976 and a "Maitrise en Science" degree in animal science in January 1978. He continued his training in France at "l'Institut National Angronomique" in Paris and at the research station of l'Institut National de la Recherche Agronomique in Jouy-en-Josas. In June of 1978, he received a "Diplome d'Etudes Approfondies" in nutrition from "l'Universite de Paris". In August of 1978, he was employed as a biologist by Agriculture-Canada at the research station in Lennoxville, Quebec. In September of 1979, he was granted a study leave by Agriculture-Canada and pursued the Doctor of Philosophy degree in animal science, with emphasis in reproductive biology under the guidance of Dr. William W. Thatcher at the University of Florida, Gainesville. In April of 1983, he was elected as an associate member of the Scientific Research Society, Sigma Xi.

Upon completion of the Doctor of Philosophy degree, he will return to Canada and serve in the position of research scientist for Agriculture-Canada at the research station of Lennoxville, Quebec.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

William W. Thatcher

William W. Thatcher, Chairman
Professor of Animal Science and
Dairy Science

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Fuller W. Bazer

Fuller W. Bazer
Professor of Animal Science

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August 1984

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